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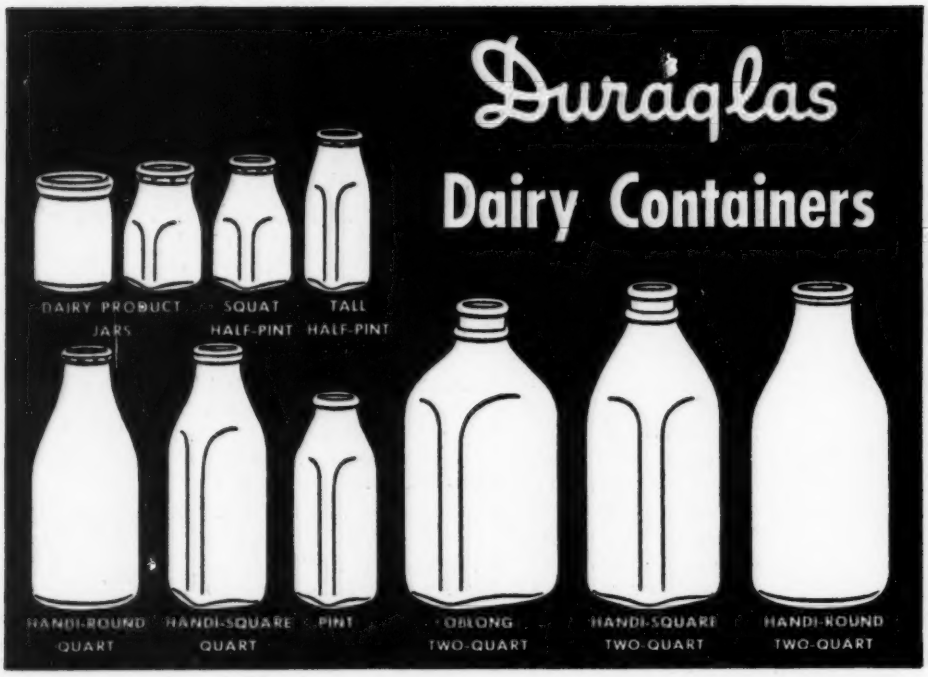

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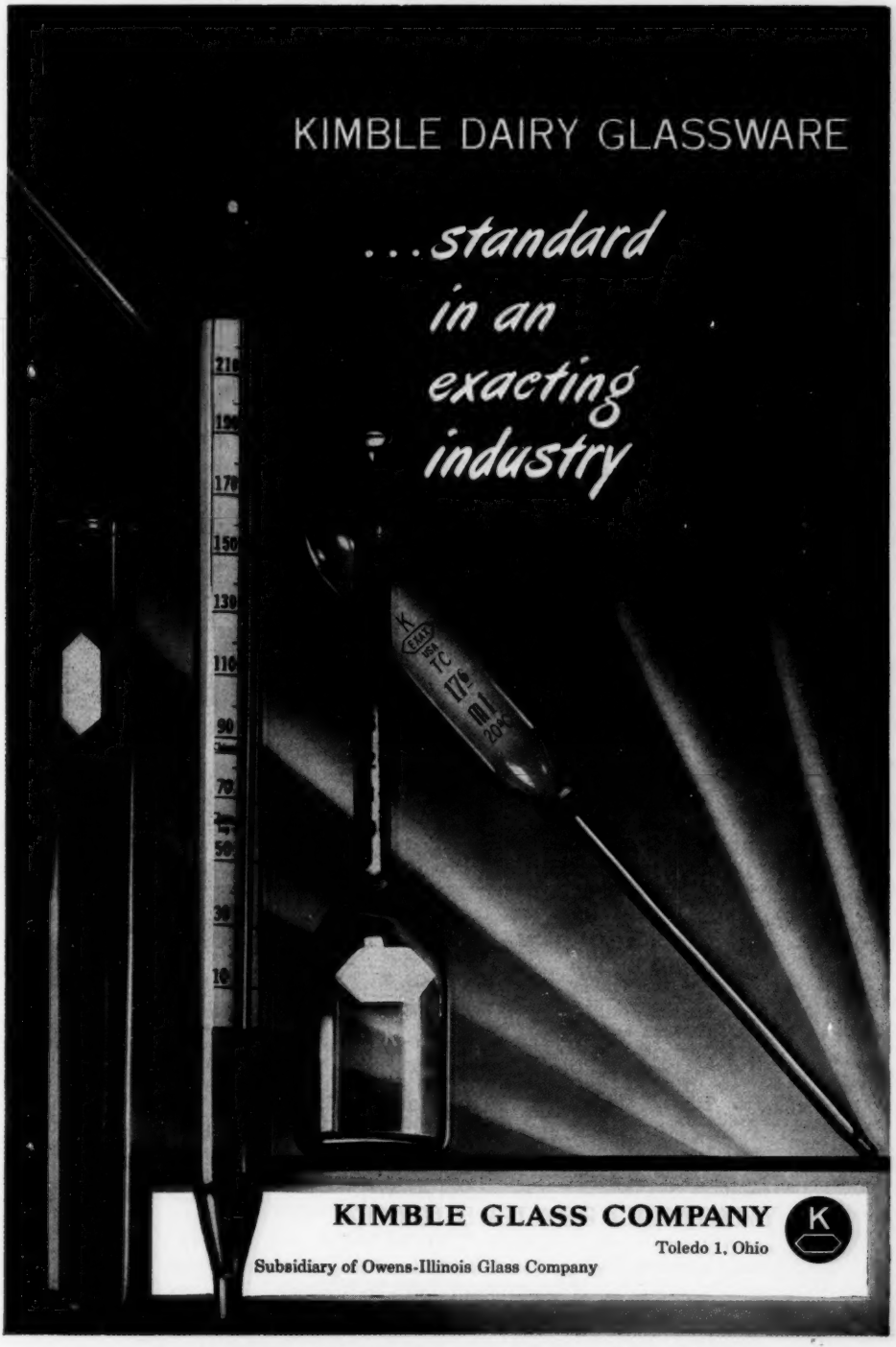

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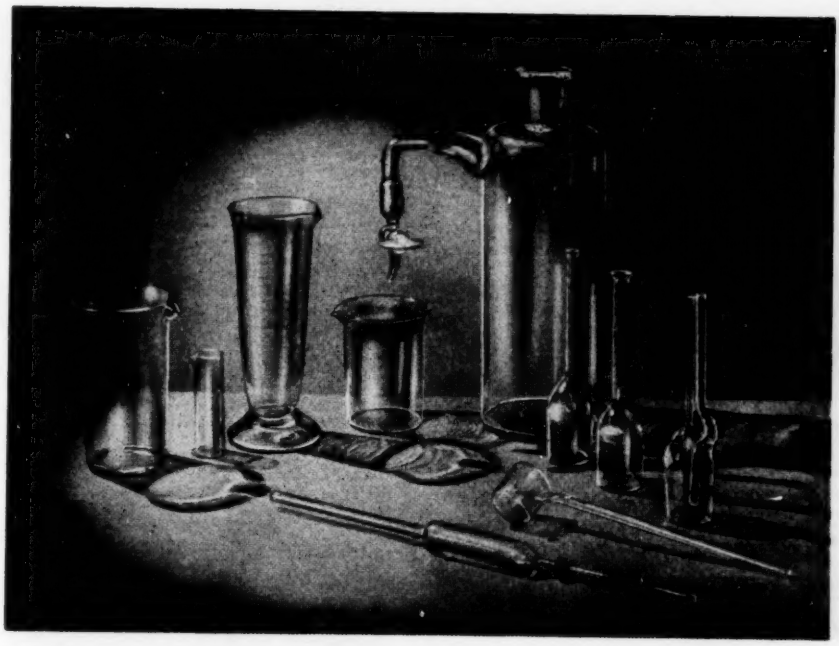

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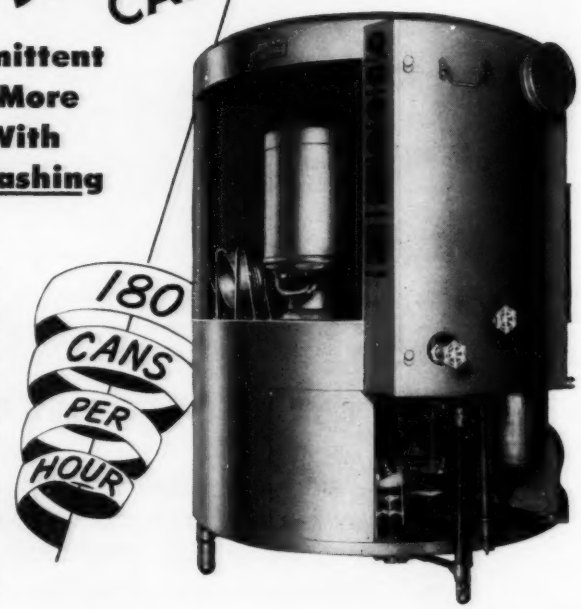

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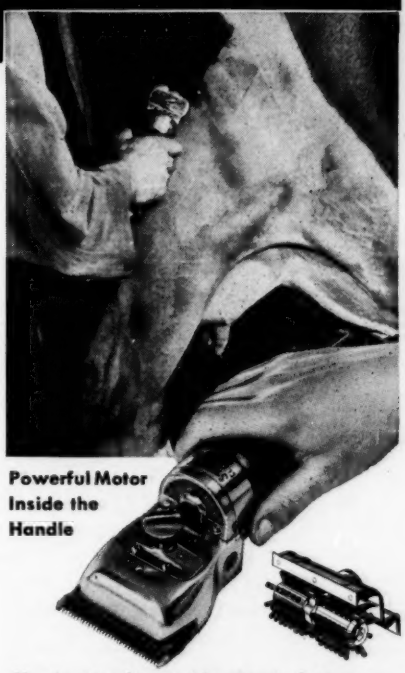

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SEMEN PRODUCTION, FRUCTOSE CONTENT OF SEMEN AND FERTILITY OF DAIRY BULLS AS RELATED TO SEXUAL EXCITEMENT[1]

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The present widespread use of the semen of a dairy bull in artificial insemination makes it necessary that his semen production be as efficient as possible. Any practice or practices that will increase his semen production, semen quality and fertility are highly desirable, particularly, if he is an outstanding sire. Observations and limited experimental data on male animals (1, 2, 3, 4, 5, 6, 11, 16, 19), as well as one objective experiment by Collins *et al.* (10) with dairy bulls indicate that sexual excitement prior to service is one of the factors which will markedly influence semen production.

Collins *et al.* (10) clearly demonstrated that restraining as compared with not restraining dairy bulls prior to semen collection with the artificial vagina resulted in highly significant increases in semen production and semen quality. However, they gave no data on the percentages of usable ejaculates and fertility of the semen. Also, these workers could not make any comparisons between first and second ejaculates, since only one ejaculate was collected each time. Since many artificial breeding organizations average approximately two ejaculates from each bull at each collection period, characteristics of first and second ejaculates as related to sexual excitement are important. Two of the most significant criteria of judgment of any practice involving dairy bulls are the efficiency of semen production and fertility of the semen.

In view of the above-mentioned lack of data in the literature, two experiments were conducted to relate semen production, fertility of the semen and fructose content of the semen to three different controlled levels of sexual excitement of mature dairy bulls. Fructose content of semen was included because of its source and role in semen metabolism as shown by Mann (13, 14, 15) and because sexual excitement was believed to be a factor influencing the level of fructose.

EXPERIMENTAL METHODS

Experiment 1. This experiment was conducted with six mature bulls, two Jerseys, two Holsteins and two Guernseys, selected from the active stud of the

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Dairy Improvement Center. The levels of sexual excitement studied were as follows: (A) Unrestraint, (B) one false mount and (C) two false mounts prior to the service of the artificial vagina. The duration of the experiment was 18 wk. (Nov. 1, 1950, through March 9, 1951). The basic experimental design consisted of 3×3 Latin squares.

The procedures for accomplishing the levels of sexual excitement for treatments A, B and C were the same as those described by Collins *et al.* (10), except that for treatment C the bulls were allowed two false mounts prior to service. In this experiment only one ejaculate was collected from each bull at each weekly collection period.

The feeding and management of the bulls was the same for all bulls. The feeding conformed to the recommended allowances of total digestible nutrients and protein by Branton *et al.* (7, 8). Since all bulls had free access to pastures averaging about 0.9 acre in size, they were not exercised on a mechanical exerciser. Throughout this study the pastures furnished very little nutrients.

The semen production and semen characteristics data were obtained by methods previously described (9). Semen samples meeting the following minimum quality standards were shipped for artificial insemination purposes: An initial motility of 50 per cent or better, a concentration of 500×10^6 spermatozoa per milliliter of semen and a modified methylene blue reduction time (240×10^6 spermatozoa per test) of 9 min. or less. The semen was diluted to contain 12×10^6 motile spermatozoa per milliliter diluted semen with egg yolk-citrate containing sulfanilamide, streptomycin and penicillin. The fructose content of the fresh, undiluted semen was determined immediately after collection by means of the Selewanoff reaction, using modifications of Roe's method (20) for blood fructose and the method for semen proposed by Mann (14). For the measurement of color density, a Klett-Summerson photoelectric colorimeter was used with a green filter (no. 54).

Experiment 2. Nine mature bulls, three each of the same breeds as above, were used in this study. The experimental design, levels of sexual excitement and other experimental methods were the same as those for experiment 1, except that during this study two ejaculates instead of one were collected at each weekly collection period. To avoid excessive irritation of the penis, each bull was subjected to the respective levels of sexual excitement for first ejaculates only. Thus, second ejaculates were collected without any restraint within a few minutes after the first ejaculates. Good pastures consisting of grasses and legumes were available to all bulls throughout most of this experiment. Consequently, little or no hay was fed.

RESULTS

Experiment 1. A summary of the mean values for all bulls on the three different levels of sexual excitement is shown in table 1. Statistical analyses of the data according to Snedecor (21) revealed that the difference between the treatment means of A and B or C for volume per ejaculate, numbers of spermatozoa, number of motile spermatozoa per ejaculate and percentages of usable ejaculates were significant ($P < 0.01$). For fructose content of the semen, differences be-

tween the means for treatment A and B were significant, and differences between B and C were significant ($P < 0.05$). However, differences between A and C were not significant for fructose content. Excluding the data from the Guernsey bull that died with a twisted cecum during the course of the experiment showed that these differences were even greater. This was the only bull that failed to show the same general trend in responses to the levels of sexual excitement.

Correlation coefficients between spermatozoa concentration and fructose content of the semen on a total, among and within-bull basis were -0.3812, -0.2178 and -0.4909, respectively. The total and within-bull correlations were significant ($P < 0.05$). The correlations between volume per ejaculate and fructose content of the semen were $+0.1805$, $+0.5961$ and -0.2261, respectively, with only the last coefficient being significant.

TABLE 1

Summary of semen characteristics, fructose content and fertility of the semen as related to sexual excitement

Criteria of responses	Treatments		
	A (Unrestrained)	B (One false mount)	C (Two false mounts)
Volume per ejaculate (*ml.*)	3.7	4.5	4.9
Initial motility (%)	58	63	61
No. sperm per ml. ($X10^6$)	1,137	1,414	1,377
No. sperm per ejaculate ($X10^6$)	4,516	6,405	6,370
No. motile sperm per ejaculate ($X10^6$)	2,562	3,977	3,890
Fructose per ml. (*mg.*)	6.1	5.4	5.9
Usable ejaculates (%)	75.8	97.0	93.9
No. first services	325	773	970
Percent 60- to 90-day non-returns	67.1	68.8	71.8

Experiment 2. The average values for this experiment are given in table 2. Statistical analyses of the data for first ejaculates showed that differences between the means for treatments A and B or C were highly significant for numbers of spermatozoa per milliliter and per ejaculate, numbers of motile spermatozoa per ejaculate, fructose per milliliter and percentages of usable ejaculates. Volume per ejaculate and initial motility differences were significant. None of the differences between treatments B and C were significant for first ejaculates. Second ejaculate treatment mean differences were not significant. When the data for first and second ejaculates were combined, the same highly significant differences between the means for treatments A and B or C were found as for first ejaculates alone. The correlation coefficients for this experiment are given in table 3.

DISCUSSION

The results of the first experiment as shown in table 1 are in agreement with those of Collins *et al.* (10), who reported that restraining bulls prior to service was accompanied by highly significant increases in semen volume per ejaculate and in numbers of spermatozoa per milliliter of semen, as well as a 41 per cent

TABLE 2

Averages for the semen characteristics, fructose content and fertility of the semen as related to sexual excitement

Criteria of responses	Ejac.	Treatments		
		A (Unrestrained)	B (One false mount)	C (Two false mounts)
Vol./ejac. (*ml.*)	1	3.6	3.9	4.0
	2	5.6	5.8	5.7
	1+2	9.1	9.7	9.8
Initial motility (%)	1	53	57	58
	2	55	54	53
	1+2	54	56	56
No. sperm/ml. ($X10^3$)	1	959	1,315	1,413
	2	1,004	1,010	1,007
	1+2	986	1,132	1,175
No. sperm/ejac. ($X10^3$)	1	3,422	5,131	5,701
	2	5,573	5,901	5,785
	1+2	8,995	11,032	11,486
No. motile sperm/ejac. ($X10^3$)	1	1,841	2,893	3,362
	2	3,102	3,322	3,112
	1+2	4,942	6,304	6,474
Fructose/ml. (*mg.*)	1	6.4	5.8	5.4
	2	6.0	6.0	5.9
	1+2	6.2	5.9	5.7
Usable ejac. (%)	1	61.1	96.3	92.6
	2	81.5	81.5	79.6
No. 1st services	1+2	960	861	1,115
% 60- to 90-d. non-returns	1+2	67.7	67.1	69.9

increase in the number of motile spermatozoa per ejaculate. In the first experiment reported herein, the average volume per ejaculate was increased by 22 per cent and the number of motile spermatozoa per ejaculate by 55 per cent when the bulls were restrained similarly (one false mount). Two false mounts re-

TABLE 3

The relationship between volume per ejaculate, spermatozoan concentration and fructose content of semen

Relationship	Correlation coefficients					
	First ejaculates			Second ejaculates		
	Total	Among bulls	Within bulls	Total	Among bulls	Within bulls
Sperm conc. and fructose content	−0.5962**	−0.7255	−0.5511**	−0.4294**	−0.4764	−0.4227**
Vol./ejac. and fructose content	+0.1099	+0.4652	−0.0425	+0.2608*	+0.4795	+0.1021

* Significant at 5% level of probability.
** Significant at 1% level of probability.

sulted in a 33 per cent increase in the average volume per ejaculate and a 52 per cent increase in the number of motile spermatozoa per ejaculate. The practical applications of these findings are clearly shown by the percentages of usable ejaculates and the number of first services for each treatment in table 1.

In the second experiment during which two ejaculates were collected at each of the 18 weekly collection periods, increases similar to those secured in experiment 1 in volume and the number of motile spermatozoa per first ejaculate and in the percentages of usable first ejaculates were obtained (table 2), whereas, the quantity and quality of second ejaculates were not affected to any extent by the level of sexual excitement prior to the first ejaculate.

Mercier and Salisbury (18) noted that the second ejaculates contained more motile spermatozoa than did first ejaculates collected immediately after bringing the bull from the barn to the collection stall. Collins *et al.* (10) pointed out that the superiority of the second ejaculates over the firsts may have been to some extent a result of increased sexual excitement. Table 2 clearly shows that their opinion is justified. First ejaculates of excellent quality can be obtained when bulls are properly restrained or excited prior to service. On the other hand, unrestraint results in many inferior first ejaculates.

The economy and efficiency of sire utilization in terms of the number of sires needed to produce a particular volume of diluted semen and in the extensive use of the semen of meritoriously proved sires can be visualized from the results presented in tables 1 and 2, particularly in the latter table. Optimum use of sires can be made when they are properly restrained prior to the first ejaculates and when two ejaculates are collected at each weekly collection period. Two false mounts appear to have little or no advantage over one false mount for most bulls. Due to the excessive irritation to the penis which may result in many instances when two false mounts are practiced and to the slight advantage of two false mounts over one false mount, the proper and recommended procedure appears to be to use one false mount prior to the first ejaculates routinely. This has been done in the Dairy Improvement Center bull stud for the past several months with very good results. However, long-time studies of the practice of using one false mount are needed before final conclusions can be drawn.

Mann (14) and Melampy *et al.* (17) reported results which indicated that there is no constant relationship between spermatozoan density and fructose concentration of the semen of bulls. Of the seven breeds studied by the latter investigators (17), only the Guernsey bulls showed a highly significant regression coefficient of fructose concentration on the volume per ejaculate. Both groups of investigators are of the opinion that the total fructose produced is directly correlated with the secretory activity of the accessory glands. However, under the conditions of experiments reported herein, fructose concentration had a very definite highly significant negative relationship with spermatozoan density and little or no relationship with volume per ejaculate (table 1, 2 and 3). A total of 99 ejaculates and 162 first and 162 second ejaculates was involved in the first and second experiments, respectively. These findings indicate that fructose concentration can be influenced markedly by the level of sexual excitement prior

to service and that as spermatozoan density increases there generally is an accompanying decrease in fructose concentration of the semen. The latter results appear to be logical in view of the fact that if the spermatozoa account for an increasing proportion of the volume of an ejaculate, a decreasing amount of fructose would be found.

SUMMARY

Three levels of sexual excitement, namely unrestraint, one false mount and two false mounts prior to the first ejaculate, were related to semen production, semen characteristics, fructose concentration and fertility of the semen of mature dairy bulls in two 18-wk. experiments. It was found that all of these criteria of responses except fertility can be influenced markedly by controlled sexual excitement. There appears to be an inverse relationship between fructose concentration and spermatozoan density of semen. The results indicate that one false mount can be recommended for routine practice by bull studs.

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A METHOD FOR THE INTRAUTERINE INSEMINATION OF CERTAIN COWS

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It has long been observed by field workers and technicians engaged in artificial insemination that the cervical folds of certain cows are in such a position that intrauterine insemination is impossible. These same cows often fail to conceive, possibly because the sperm are not able to pass such a hindrance. Furthermore, cervicitis and abnormal cervical secretions can, in themselves, be causes of infertility. This has been shown to be true in humans (4, 6) and Roark and Herman (5) have shown that it is probably the case with cattle also.

In view of these facts it may seem advisable, in certain cases where the cervix is causing difficulty, to by-pass this structure in insemination. A technique has been developed whereby this is made possible. A similar technique has proved successful in mice (3).

EXPERIMENTAL PROCEDURE

The method consists of manually directing an open needle, with tube attached, through the rectum to a point over the uterus 2 to 5 in. forward from the anterior end of the cervix. The needle is pushed through the rectal wall into the peritoneal cavity and, in turn, into both horns of the uterus. The semen is deposited by means of a syringe on the end of the tubing which protrudes from the anus.

The equipment used was simple in construction and parts were employed which are easily obtainable. Two ft. of three-sixteenths in. tygon tubing were used as the connector from needle to syringe. The portion above the shoulder of a 16-gauge California bleeding needle was broken off and the shoulder of the needle forced into one end of the tube, forming an airtight connection and leaving the sharp end of the needle protruding. A 30-ml. Luer-loc syringe was attached to the tubing by means of an adapter with an expanded shoulder on the end which was inserted into the tube. This made an airtight connection on the syringe end. Airtight connections were essential in order to prevent the semen from being lost before the puncture was made. A picture of the equipment is given in figure 1.

When a cow was to be bred, the equipment was assembled and rinsed first with alcohol and then distilled water. After the equipment was dry, the plunger of the syringe was drawn up to the 15-ml. mark on the barrel and 3 to 5 ml. of raw semen were pulled into the lower end of the tube. At this point there were 18 to 20 ml. of air in the syringe. The extra 15 ml. of air were needed to com-

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pletely flush the semen from the tube. The semen was not drawn into the syringe because this procedure would require the insertion of more air into the uterus than was necessary.

When the semen had been drawn into the lower end of the tube, a cork was put over the sharp end of the needle to completely seal the system, and so to keep the plunger of the syringe from slipping and to decrease contamination caused by fecal accumulation in the needle. The cork also kept the needle from puncturing the rectal wall while traversing the rectum.

After excess fecal material was removed from the rectum, the uterine horns were located. With one hand the operator manually inserted the needle into the rectum and with the other he held the syringe. The syringe was held higher than the cow's anus so that the semen would not travel toward the syringe, but would be held by gravity against the needle.

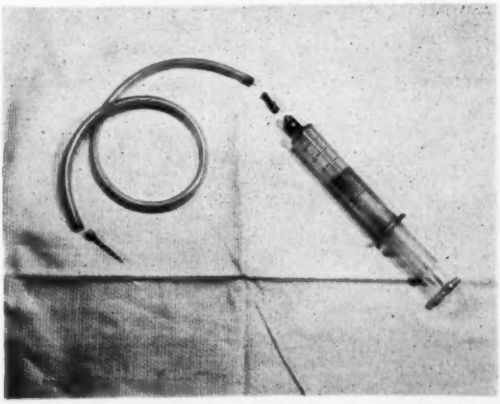

FIG. 1. Equipment used for the technique described.

By grasping the tube just above the shoulder of the needle, with the thumb and first two fingers of the inserted hand, slipping of the fingers along the tube or needle could be practically eliminated when pressure was applied. The third finger was used as a probe to locate the spot where the needle was to be inserted. When the needle was pushed through the rectal wall at the point where the third finger had been feeling the uterine horn, the horn was easy to locate and rather easily penetrated when the end of the needle was in the peritoneal cavity. When the needle was inserted straight down and the horn punctured close to a point on the horizontal plane and directly on top, the lumen of the horn would, in most cases, be entered the first time. When the needle failed to enter the lumen and the end of the needle was in the uterine wall, there was back pressure on the syringe and the semen could not be expelled. Care was taken not to go through the lumen and thereby expel the semen into the peritoneal cavity ventral to the uterus. When it was judged that the end of the needle was in the lumen, half of the semen was expelled. The process then was repeated in the other horn. The needle was withdrawn into the rectum and a new puncture made through

the rectal wall in order to lessen the strain on the wall and reduce the possibility of tearing it. Figure 2 shows, diagramatically, how the technique was performed.

To determine the physical damage to the uterus (by hematoma or trauma) three cows were slaughtered within 3 hr. after the new method had been employed. In the practice of the technique on these three cows, milk of magnesia was used instead of semen so that it could be determined whether or not a given substance actually could be injected into the uterine horns. Milk of magnesia was the substance chosen because it is easily detected and is not absorbed.

The same three cows which were slaughtered had been used as experimental subjects on which the technique had been perfected. Each had been subjected to the treatment a minimum of two times, over a period 4 mo. previous to slaughter. Therefore, it was thought that if the operation were to introduce a chronic infection, it would be apparent after this length of time.

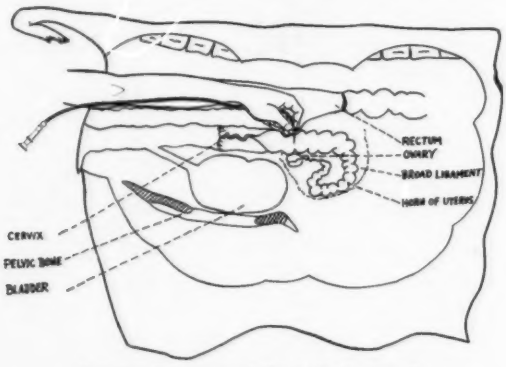

FIG. 2. Diagram of the technique used.

Of the six horns in the three cows which had been injected with milk of magnesia, the substance was found in four of the horns when the animals were slaughtered. On the remaining two, external signs of probing were in evidence, but the lumina of these horns were small and were not successfully injected. The loci of entrance into the four horns in which the operation was successful were from 2 to 5 in. anterior to the cervix. They were detected easily on both the interior and exterior of the uterine wall by small areas of petechiation. The areas ranged in size from 0.5 to 2 mm. in diameter.

To ascertain if any acute infection developed due to the relatively high bacterial contamination which necessarily prevails in the practice of this technique, total leucocyte counts were made on all cows which were so bred both immediately before and 48 to 72 hr. afterward. Any acute infection should be indicated by leucocytosis.

Table 1 shows the leucocyte count in number of cells per cubic millimeter, both before and 48 to 72 hr. after the technique had been employed on each of the cows. When these data were analyzed by the *t*-test method for paired com-

parisons, as described by Snedecor (7), there was found to be no significant difference between the two groups.

The average count on all cows before treatment was 7,980, while the average count after treatment was 7,935. The average leucocyte count per cubic millimeter given by Dukes (1), for the ox is 7,900. This indicates that no damage to the uterus or associated organs resulted which would cause abnormal systemic reaction.

During the course of this experiment, four cows were inseminated. One of these, 11S, was used because she had been culled from the University herd after having been bred ten times both naturally and artificially without resultant pregnancy. When she had been dried off at the end of her lactation, she was used by

TABLE 1

Total leucocyte count in cells/mm.³ before and after treatment

Cow no.	Date	Before treatment	After treatment	Difference
11S	5/3	9,430	7,930	−1,500
	7/3	6,200	8,200	+2,000
	8/25[a]	5,500	6,050	+ 550
321G	5/3	9,210	10,500	+1,290
	9/29	7,600	6,900	− 700
J1	7/9	8,350	6,450	−1,900
J2[b]	7/9	12,550	8,600	−5,850
J3	7/12	11,500	9,425	−2,075
	7/27	9,000	9,750	+ 750
	8/14	9,900	9,900	0
J4	7/12	8,250	8,100	− 150
	7/27	5,400	7,500	+2,100
	8/14	5,200	6,000	+ 800
511H	8/21[a]	7,900	8,400	+ 500
367A	9/7[a]	5,900	6,400	+ 500
348A	9/29[a]	5,800	5,950	+ 150
Av.		7,980	7,935	

[a] Cow bred on this date.
[b] This cow was about 180 d. pregnant at this time.

the blood antigen laboratory as a source of blood for 6 mo. At the end of this period, she was used as a source of blood for other work and an average of 1,000 ml. was drawn from her weekly. When it was decided to breed her by the method herein described, she had not been observed in heat for 3 mo., although her ovaries were functional and other parts of her reproductive tract appeared normal. Heat was induced by manual massage of the ovaries and she was bred during her first period.

The second and third cows bred were 367A and 348A. They were first-calf heifers culled from herd for low production. Neither had been observed in heat since freshening 3 mo. previously and 367A had to be induced to heat by manual massage of the ovaries. Cow 348A had only a few of the normal physiological symptoms of heat when she was inseminated.

Number 512H was the fourth cow on which the method was attempted. This

animal was included in the experiment after having aborted her first calf at 6 mo. She was blood tested for brucellosis and found to be negative.

RESULTS AND DISCUSSION

Three of the cows previously discussed, 11S, 348A and 367A, have all dropped living, healthy calves. The gestation lengths were 301, 275 and 277 days, respectively. The mean lengths of gestation for Brown Swiss and Ayrshires are 289 and 278 days, respectively (2).

Both the autopsy and blood count data indicate that no physical or physiological damage was done to the uterus or associated organs. The small areas of petechiation were not considered large or extensive enough to be of any consequence in themselves, nor did they appear dangerous as potential loci of infection.

In the cows which were slaughtered, there was no visible sign of peritonitis or metritis, even though the animals had been used as subjects on which the technique was practiced for 4 mo. previous to slaughter.

Limited field trials are now under way using this technique on difficult breeding cows whose case histories indicate cervical difficulties.

SUMMARY AND CONCLUSION

A method for the intrauterine artificial insemination of cattle whose cervices cannot be penetrated is described. Evidence is presented to show that physical and physiological damage caused by the technique on nine cows in the experiment was negligible. Delivery of a living calf by three of the four cows inseminated but one time by the new method is reported. Its place in the over-all artificial breeding program is considered to be of value on certain types of difficult breeding cows which would otherwise be discarded.

ACKNOWLEDGMENT

The authors are indebted to H. A. Herman and F. W. Madden for their kind permission to use figure 2, the basic outline for which came from their book, "The Artificial Insemination of Dairy Cattle, A Handbook and Laboratory Manual," published by Lucas Bros., Columbia, Mo., 1949.

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THE CARRIER STATE OF LACTIC STREPTOCOCCUS BACTERIOPHAGE[1]

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Presence of bacteriophage in some apparently normal multiple-strain, commercial lactic cultures was observed by Nelson *et al.* (4), but they did not explore the background for this phenomenon. More recently workers in this laboratory have recovered from a number of apparently normal commercial lactic cultures bacteriophage active against several stock cultures of lactic streptococci previously isolated from various sources. The possible implications of dissemination of bacteriophage by sending into dairy plants cultures in which bacteriophage is present prompted the studies which are reported in this paper.

Bacteriophage occurring in regular and intimate association with cultures of the host bacteria but not causing noticeable delay in growth of the culture apparently was first observed by Lisbonne and Carrére (3) in cultures of *Bacterium* (*Escherichia*) *coli*. The characteristics of lysogenesis, as the phenomenon was called, were summarized by Flu (1) as (a) production of bacteriophage by every cell in a given culture, (b) failure of the bacteriophage to cause lysis of the host cell and (c) inseparability of the bacteria from the bacteriophage. Work by Hunter (2) indicates a "carrier" state in the association between lactic streptococcus bacteria and bacteriophage. This state differs from lysogenesis in that the bacteria are easily separated from the bacteriophage by a simple plating and re-isolation procedure. Reiter (10) has demonstrated what he considered true lysogenic strains of lactic streptococci. Pette (8) also has described a similar situation.

METHODS

The mixed cultures under study were maintained in non-fortified skimmilk, and all pure culture indicator strains were maintained in litmus milk with 10 per cent filtered V-8 juice[4] added. Cross-reaction tests for bacteriophage action upon bacteria were made in litmus milk fortified with 10 per cent V-8 juice, 0.2 per cent glucose and 0.5 per cent trypticase.[5] Bacteriophage numbers were estimated with the limiting dilution technic, using the triply fortified litmus milk medium, the number of tubes used depending upon the precision desired. Determinations of bacterial populations and isolations of representative strains were made by plating the cultures on tomato juice agar.

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[4] A combination of eight vegetable juices made by the Campbell Soup Co., Camden, N. J.

[5] A pancreatic digest of casein made by Baltimore Biological Laboratories, Inc., Baltimore, Md.

Bacteriophage filtrates were prepared by filtering the culture, previously coagulated with sterile lactic acid if normal coagulation had not occurred, through sterile coarse filter paper and then filtering the resulting whey through a Selas porcelain filter of #03 porosity. Filtrates prepared from the mixed cultures at various transfers were designated with the number of the transfer from which they were prepared and the designation of the culture preceded by the letter *f;* for example, the filtrate from transfer 16 of culture B was termed fB16. Single-plaque isolations sometimes were made from these filtrates and the letter *P* then was added for designation of the subsequent purified filtrates; PfB16 indicates purified filtrate from transfer 16 of culture B. Bacteriophage strains designated by an *F* prefix were from the Laboratory collection.

RESULTS

Bacteriophage (filtrate F80; titer 10^4) was demonstrated in culture A_1, a commercial multiple-strain culture which had been propagated in the laboratory six times per week for a period of 2 yr. This culture had been slow several times when used in making cottage cheese during the months immediately preceding demonstration of bacteriophage, but lack of proper single-strain test cultures had prevented immediate detection of the bacteriophage. Transfers from the original culture had been frozen the fifth day (culture A_3) and also about 1 yr. after receipt (culture A_4), and these frozen cultures were available for the studies. Culture A_2 was from the same original culture as A_1 but had been transferred at weekly intervals. A new culture (A_5) from the same source was acquired almost a year after the original culture had been obtained; this culture was transferred weekly for 1 yr. before examination. A second new culture (A_6) was acquired 2 yr. after the original culture and bacteriophage (filtrate F81) was recovered at the first transfer after receipt from the commercial source. This bacteriophage was considered to have been present in the culture when received, as every reasonable precaution was taken to exclude chance contamination in the laboratory. Bacteriophage was not demonstrated in the first transfers after thawing of cultures A_3 and A_4 or in cultures A_2 and A_5 at the time of examination. Whether these four cultures ever had contained bacteriophage was not known, for no tests had been made on the original culture from which all of these were descended or on the preceding propagations. Filtrates F78, F79 and F82 were from commercial vats of slow cottage cheese; two were from cheese set with cultures from a different source than the A series. The five filtrates all were active against one or more of the isolates from culture A_1.

Marked differences in the bacteriophage sensitivity patterns of lactic streptococcus strains isolated at essentially the same time from the various cultures are shown by the percentage figures in table 1. The comparative resistance of isolates from cultures A_1, A_3 and A_5 is in considerable contrast to the relatively high percentages of isolates from A_2 and A_4 which were sensitive to bacteriophage filtrates F78 and F81. Culture A_1 was carrying bacteriophage when the isolations were made, and this may explain the low level of isolates sensitive to any of the three bacteriophage strains used for testing. The high level of sensitive

bacteria in A_2, in spite of this culture being from the same original source as A_1, may be attributable to the absence of bacteriophage in A_2. The isolates labelled A_1^a were from an earlier transfer of A_1 and show the most marked sensitivity encountered to this group of test bacteriophages.

When only 20 calculated bacteriophage particles of F78 were added to 400 ml. of sterile milk freshly inoculated with 1 per cent of culture A_2, normal coagulation occurred after 16 hr. at 21° C. However, the sensitivity pattern of isolates from the culture changed abruptly from 99 per cent sensitive to F78 and none sensitive to F79 and F80, to completely non-sensitive to F78, 17 per cent sensitive to F79 and 5 per cent sensitive to F80. Before bacteriophage F79 was added in the same manner after the ninth transfer, none of the isolates was sensitive to any one of the three bacteriophage filtrates. The titer of F78 declind from 6×10^9 to 6×10^7, 1.4×10^5, 1.3×10^3, 60, 0.2 and 0 in the second to eighth transfers, respectively. This rate of disappearance can be explained by simple serial dilution, as 1 per cent of inoculum was employed in each transfer. The titer of F79 increased very slowly after initial addition and remained at relatively low level,

TABLE 1

Percentage of bacteriophage-sensitive isolates from various cultures (100 isolations from each culture were tested)

Bacteriophage test filtrates	Bacteriophage sensitive isolates from culture:					
	A_1	A_2	A_3	A_4	A_5	A_1^a
	(%)	(%)	(%)	(%)	(%)	(%)
F78	1	83	0	43	0	62
F79	1	0	0	5	0	5
F80	0	0	9	2	0	10
F81	.....	.....	0	31	.....	61
F82	.....	.....	.....	.....	.....	5

[a] Isolated from culture A_1 but 5 mo. earlier than the A_1 isolates.

being 0.2, 1.3, 1.1×10^2, 5×10^3, 7×10^2, 1.3×10^4 and 3.5×10^3 after the tenth to the sixteenth transfers, respectively. Apparently, A_2 had had the ability to carry bacteriophage of the type of F79, but either had not been contaminated at first or had lost the bacteriophage at some earlier stage.

When bacteriophage filtrates F78, F79, F80, F81 and F82 were added together, each as 1 ml. of material with a titer of 10^7 to 10^9 per milliliter, to culture A_2 and the culture incubated at 21° C. for 16 hr., there was no appreciable drop in acid production. However, the sensitivity pattern of the culture changed from 92 per cent of the isolates sensitive to at least one of the five test bacteriophages to none sensitive.

Multiple-strain culture B, obtained from a source different from that of culture A, contained bacteriophage in the first transfer of the commercial powdered culture. The bacteriophage titers of the filtrates at each of the transfers varied from 10^2 on the first few transfers up to 10^5 for many of the transfers from the 6th through the 29th and then were mostly 10^1 and 10^2 through the 62nd transfer, dropping to 10^0 in the 63rd, 64th and 65th transfers. After transfer 65, bac-

teriophage was not demonstrable in filtrates from this culture. The titratable acidities of the culture ranged from 0.68 to 0.82 per cent after the second transfer, one transfer dropping to 0.66 per cent. Although some variations in titratable acidity values and bacteriophage titers occurred, apparently there was no direct relationship between these two characteristics.

As shown in table 2, the bacteriophage infection in culture B consisted of three somewhat overlapping phases. In phase A (filtrates fB1 and fB2) bacteriophage activity was detected with several indicator strains, most of which were cultures of the 122 or 146 series. In phase B (filtrates fB3 to fB34) definite bacteriophage activity was demonstrated against cultures Hl-2 and Hl-3. In phase C (filtrates fB34 to fB65) the bacteriophage present was active against cultures 5 and 6 only, except for filtrates fB34, fB35 and fB36, which were in-

TABLE 2

Cross-reaction patterns of typical bacteria-free whey filtrates from various transfers of culture A

Organism indicator strain	Action of bacteriophage filtrate:										
	fB1	fB2	PfB2	fB3	fB5	PfB5	fB33	fB34	fB37	fB65	fB66
Hl-2	–[a]	?	–	+	+	+	+	+	–	–	–
Hl-3	–	?	–	+	+	+	+	+	–	–	–
Hl-10	+	+	+	?	–	–	–	–	–	–	–
122-1	–	–	+	–	–	–	–	–	–	–	–
122-2	+	–	+	–	–	–	–	–	–	–	–
122-3	–	–	+	–	–	–	–	–	–	–	–
122-4	–	+	+	–	–	–	–	–	–	–	–
122-5	+	+	+	–	–	–	–	–	–	–	–
146-1	–	+	+	–	–	–	–	–	–	–	–
146-2	+	–	+	–	–	–	–	–	–	–	–
146-3	–	+	+	–	–	–	–	–	–	–	–
146-4	+	–	+	–	–	–	–	–	–	–	–
146-5	+	–	+	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	+	+	+	–
6	–	–	–	–	–	–	–	+	+	+	–

[a] – = no activity; + = active; ? = questionable.

hibitory not only to cultures 5 and 6 but also to Hl-2 and Hl-3. Possibly this resulted from residual bacteriophage activity of the type encountered in phase B, while the type found in phase C was becoming established. However, it is unknown when activity against cultures 5 and 6 appeared, since no routine checks of filtrates fB6 to fB33 against cultures 5 and 6 were made.

Representative groups of 50 cultures each were isolated from various transfers of culture B and tested against a large number of different bacteriophage races. Most of these tests gave negative results; therefore, only the reactions with 13 strains of bactriophage which most frequently gave positive results are summarized in table 3. Considerable fluctuation in the proportion of strains sensitive to different bacteriophage races was apparent, particularly during the first eight transfers; after this there was a definite decrease in the proportion of isolates sensitive to the bacteriophage strains used for testing. The possibility of adaptation of at least one of these bacteriophage strains to attack previously re-

sistant cultures clearly was demonstrated with F75, which, although originally active against only three of the fifty B-16 isolates, could be made active against all of the B-16 isolates by laboratory propagation on one of the three originally sensitive isolates.

Four additional cultures from other commercial sources were examined and bacteriophage could be demonstrated from only one of these, culture C. Sensitivity to various strains of bacteriophage was demonstrated among isolates from all four of the cultures examined.

Several attempts were made to eliminate bacteriophage from carrier cultures by initiating complete lysis of the sensitive strain supposedly serving as substratum for the associated bacteriophage or by preventing bacteriophage prolif-

TABLE 3

Cross-reactions of isolates from various transfers of culture B

Bacteriophage filtrate	Per cent of sensitive isolates[a] from transfer number								
	1	2	3	5	8	12	16	42	71
F4	12	14	2	0	2	0	0	0	0
F20	8	6	2	2	0	0	0	0	0
F26	2	10	2	4	12	0	0	0	0
F49	0	14	0	8	2	0	0	0	0
F79	16	20	20	8	4	0	0	2	0
F82	74	28	54	44	60	0	0	2	0
PF82	54	4	24	24	4	0	0	2	2
fB2[b]	12	18	10	8	0	0	0	0	0
PfB2	6	0	0	0	2	0	2	0	0
PfB5	6	0	0	0	0	0	0	0	0
F42	6	0	–	–	–	–	0	–	–
fB60[c]	0	–	–	–	–	–	0	0	0
F75	0	–	–	–	–	–	6	16	0
	Per cent of isolates not sensitive to any of the filtrates tested								
	18	50	42	25	22	100	92	80	98

[a] Fifty isolates selected from each transfer.

[b] Filtrates designed *f* or *Pf* were from the indicated transfer of the mother culture; all others were from the laboratory collection.

[c] Propagated once on culture 6 to secure titer $> 10^4$/ml.

eration, using such methods as massive additions of homologous bacteriophage (7), periodic neutralization of the culture during incubation so as to maintain the medium at a reaction near the optimum for lysis during the entire incubation period (6), and propagation of the culture in essentially calcium-free medium (9). None of these methods was successful when tried on several carrier cultures.

Attempts to recover bacteriophage from lactic streptococci isolated from culture B were made by using individual isolates selected at random and various groups of ten or 50 isolates each. The failure to recover bacteriophage from any of the groups or the individual isolates suggested that none of these strains isolated from the phage-carrying mixed culture was lysogenic, and that possibly the bacteriophage in culture B was supported by a carrier strain (2), which would be separated from the bacteriophage by plating in the process of isolation of the bacteria.

To determine the possible combinations of sensitive and resistant bacteria which might be present in carrier cultures, population studies were carried out. In these trials, milk was given a standard inoculum of sensitive and non-sensitive bacteria in proportions ranging from 1:1 to 1:10,000, plus a small amount of bacteriophage (table 4). Resistant bacteria of various origins were used in different trials. After incubating the cultures for 16 hr. at 32° C., filtrates were prepared and titered against the sensitive strain. Results of these studies indicated that a combination of slightly more than 10^3 sensitive and 10^6 resistant bacteria per milliliter of original inoculum produced in the experimental cultures bacteriophage titers similar to those commonly found in commercial cultures

TABLE 4

Suppression of bacteriophage proliferation by resistant bacteria of various origins

Trial no.	Initial number/ml.[a] of:			Final bacteriophage titer/ml. (16 hr.)
	Sensitive bacteria	Resistant bacteria	Bacteriophage particles	
I	62×10^5	none	9.5×10^1	4.5×10^8
Using a	31×10^5	42×10^5	3.0×10^1	9.5×10^8
resistant	62×10^4	75×10^5	4.5×10^2	9.5×10^7
laboratory	62×10^3	83×10^5	9.5×10^1	1.4×10^7
strain	62×10^2	83×10^5	2.5×10^2	2.5×10^4
	70×10^1	83×10^5	2.5×10^1	2.5×10^1
	none	83×10^5	4.0×10^1	4.5×10^1
II	67×10^5	none	4.5×10^1	9.5×10^8
Using a	34×10^5	35×10^5	6.0×10^1	1.5×10^8
resistant	67×10^4	63×10^5	2.5×10^0	7.5×10^7
secondary-	67×10^3	70×10^5	2.5×10^0	4.5×10^5
growth	67×10^2	70×10^5	2.5×10^1	1.5×10^2
strain	80×10^1	70×10^5	2.5×10^1	2.5×10^0
	none	70×10^5	4.0×10^1	2.5×10^0
III	62×10^5	none	9.5×10^1	4.5×10^8
Using a	31×10^5	40×10^5	4.5×10^1	9.5×10^7
resistant	62×10^4	73×10^5	9.5×10^1	2.5×10^7
"wild"	62×10^3	81×10^5	4.5×10^1	4.5×10^6
S. lactis	62×10^2	81×10^5	4.2×10^2	4.5×10^5
strain	70×10^1	81×10^5	2.5×10^1	4.0×10^1
	none	81×10^5	2.5×10^1	4.5×10^0

[a] Bacterial numbers were estimated by plating the dilutions from which the 1st and last inoculations were made.

which carried bacteriophage (10^2 to 10^5 per milliliter). Upon the basis of these data, a carrier culture could contain sensitive bacteria in such small numbers that probably none could be picked when only 50 to 100 isolations were made. In all cases, bacteriophage proliferation definitely was suppressed in the presence of a large proportion of non-sensitive bacteria.

Experimental culture E_1 similar to that used in trial I of table 4 was prepared and observed for a number of propagations at 32° C. The bacteriophage titers, determined with single tubes of milk at each dilution, of the filtrates from the first 48 transfers of culture E_1 are shown in figure 1. The culture carried the bacteriophage during the entire observation period, and the outstanding feature was the marked fluctuation in titer. Although bacteriophage often was

present in concentrations as high as 10^7 per ml., the culture appeared normal and showed no indication of bacteriophage inhibition. The changes in bacteriophage titer tended to follow a cycle in which periods of rapid increase were followed by periods of rapid decrease in population, indicating a dynamic equilibrium between bacteriophage and sensitive bacteria. A control culture to which no bacteriophage was added was carried for six transfers with no indication of bacteriophage in any of its filtrates. When bacteriophage was added to the seventh transfer of what had been the control culture, bacteriophage was retained throughout an observation period of 25 transfers at levels almost identical with those observed in culture E_1.

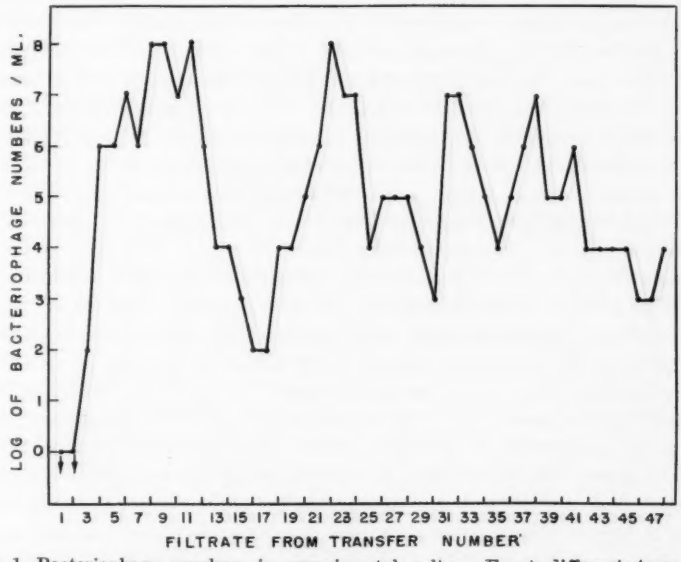

FIG. 1. Bacteriophage numbers in experimental culture E_1 at different transfers.

DISCUSSION

Certain commercial cultures used for production of lactic acid and flavor compounds in various dairy processes carry bacteriophage. Failure to demonstrate lysogenic strains among isolates from these cultures suggests another mechanism of bacteriophage maintenance, probably the carrier state described by Hunter (2). The data obtained definitely indicate that mixtures in the right proportions of resistant bacteria and normal, non-lysogenic, sensitive bacteria support limited proliferation of bacteriophage through a considerable series of transfers without the appearance of an observable abnormality in the mixed culture. This evidence suggests an equilibrium between sensitive and resistant bacteria in commercial cultures carrying bacteriophage. However, the probable low level of the sensitive bacteria almost precludes their detection by normal isolation procedures.

Several factors probably are important in maintaining an equilibrium which permits continuing bacteriophage proliferation at a low level which does not seem to affect acid production by the culture. Overcast *et al.* (6) have shown that shifts toward acid reaction markedly prolong the lag phase of lactic streptococcus bacteriophage proliferation. When relatively heavy inocula, such as were used in these studies and are in common use in industrial handling of lactic cultures, are employed, the pH may be expected, because of growth of the resistant bacteria, to drop rather rapidly into a range which markedly retards bacteriophage proliferation. Such a culture may reach a physiological state completely unsuited to bacteriophage proliferation considerably before mass lysis of the sensitive strain occurs. The data of table 4 show that large numbers of resistant bacteria in the presence of relatively few sensitive organisms have a very marked inhibitory action on bacteriophage increase. There is the possibility that, at least in some cases, some bacteriophage particles might be adsorbed to resistant cells and thus not reach the sensitive bacteria. If any considerable portion of the particles was removed in this manner, a marked slowing of proliferation could result from this factor alone. There might be competition between the sensitive and the resistant bacteria for a nutrient essential to bacteriophage increase, although this explanation might not be valid under many circumstances when a complex medium such as milk is being used.

An explanation of the appearance in sequence of three different bacteriophage types in culture B is not apparent from the data available. Appropriate precautions against contamination were taken and the lack of relationship among the bacteriophage types appearing in culture B and other strains of bacteriophage in regular use in the laboratory does not suggest contamination of the culture.

Distribution of sensitive and non-sensitive strains of bacteria is not constant from transfer to transfer of the same mixed culture. Presence of bacteriophage shifts the culture very quickly to a predominance of bacteria not sensitive to that bacteriophage strain. There are indications that other factors related only incidentally to bacteriophage sensitivity may be operative, since changes also occur in the absence of bacteriophage. This might be expected since sensitivities to antibiotics, quaternary ammonium compounds, adverse temperatures and many other factors seem to be independent of bacteriophage sensitivity. This is of practical importance, for a mixed culture in which one strain had become dominant might behave much as would a single-strain culture, since infection with a bacteriophage active against the dominant strain would result in almost complete stoppage of acid production. Were this same organism strain only a minor fraction of the mixture, infection with the same bacteriophage strain might go undetected, because non-sensitive strains would be present in large numbers to carry out the fermentation essentially normally.

The carrier state in commercial lactic cultures may be of practical importance as a means of introducing bacteriophage into a plant. The bacteriophage would not cause trouble in the culture in which it was carried at the time of introduction. However, it would be possible for the culture population to shift at a later date (probably after the culture had become free from bacteriophage, as happens spontaneously in some instances) to a predominance of sensitive organisms which

could be lysed by the reintroduction of the bacteriophage. The comparatively long periods during which bacteriophage may persist on equipment or structure or in products in the plant would make reintroduction from these sources fairly easy. A new culture introduced subsequently might be sensitive to the old bacteriophage previously introduced. The possibility exists, as Nichols and Hoyle (5) have shown, that the bacteriophage strain might adapt to attack strains of bacteria previously resistant and thus be responsible for lysis of a culture not attacked previously. It is only fair to state, however, that none of these possibilities has been demonstrated in plant practice, although that does not mean that these situations may not have arisen; they might easily have passed undetected, for complete histories are lacking on most bacteriophage outbreaks.

Bacteriophage carried in a multiple-strain commercial lactic culture might easily be responsible for the continued holding at a very low level of one or more of the bacterial strains, resulting in a predominance of one or more other strains. If a bacteriophage active against the dominant strain(s) were introduced, the result would be much more complete disruption of acid production than would have been the case had several strains of different bacteriophage sensitivities been present in essentially equal numbers.

Presumably the way in which considerable certainty of freedom from bacteriophage could be assured at the present level of knowledge is the periodic testing of filtrates from the culture against a collection of strains of lactic streptococci known to be sensitive to a wide variety of bacteriophages. Lack of the proper test organism would lead to the erroneous conclusion that the culture was free from bacteriophage. Possibly the maintenance of the component organisms of the culture as pure strains to be combined periodically would simplify detection of bacteriophage. However, one would need to run tests to determine that the individual strains were neither lysogenic nor carriers. Also there would be the problem of attaining satisfactory balance of the various strains in each recombination attempted.

SUMMARY

Certain normally developing multiple-strain cultures of lactic streptococci found to carry bacteriophage have been investigated. The evidence suggests that there exists in these cultures an equilibrium between sensitive bacteria, resistant bacteria and the associated bacteriophage, resulting at each transfer in incomplete lysis of the sensitive strain, thereby continually providing substratum for the associated bacteriophage.

In a multiple-strain culture, the numerical relationships between the various strains are not stable; the relationship may change considerably from one transfer to the next. Presence of bacteriophage is one of the factors which may cause this type of shift.

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MILK SERUM PROTEINS. I. A QUANTITATIVE BIURET TEST FOR MILK SERUM PROTEINS[1]

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The biuret reaction has been used for many years as a qualitative test for the presence of proteins in solution. It depends on the formation of a violet copper-protein complex in alkaline $CuSO_4$ solution. This reaction first was adapted as a quantitative test for protein by Autenrieth (1, 2), who determined the albumin and globulin in urine, ascitic fluid and blood serum.

A number of modifications of the biuret test have been suggested, primarily to obtain solutions free of turbidity and to increase the sensitivity of the test. Kingsley (7) recommended concentrations of 9 per cent NaOH and 0.1 per cent $CuSO_4$ in the test solution. He reported that the color complex was fully developed in 30 min. and remained stable until turbidity appeared in about 1 hr. Later, Kingsley (8) recommended centrifugation to obtain clear tests. Robinson and Hodgen (13) altered the reagent to 3 per cent NaOH and 0.5 per cent $CuSO_4$, and centrifuged and filtered to obtain a clear solution. Mehl (9) found that 0.05 per cent Cu was needed to give an excess for plasma protein concentrations up to 0.15 per cent.

Weichselbaum (14) and later Wolfson *et al.* (15) used a reagent similar to Fehling's solution and did not comment on trouble with turbidity. Trials in this laboratory have shown that the reagent used by Weichselbaum caused turbidity and was not sensitive to low concentrations of milk serum protein.

A quantitative biuret test has been reported by Molnar (12) for total protein in milk and in mixtures of milk and egg albumin. In this test the proteins are precipitated with trichloroacetic acid, centrifuged, the supernatant decanted and the proteins put into solution with alkali. Color is developed in this solution by addition of $CuSO_4$. The method is satisfactory for protein concentration from 1 to 15 per cent. Below 1 per cent the color intensity is very low and the values range much below those obtained by the Kjeldahl method.

Although the biuret method is widely used in serum protein analysis, the method has not been standardized and difficulty with turbid solution still is experienced. The purpose of this study was to develop a simple, sensitive biuret method for the measurement of milk serum proteins during fractionation.

METHODS

By milk serum proteins is meant those proteins other than casein in cow's milk; they sometimes are called whey proteins. The milk serum proteins for this work were prepared from skimmilk. First the casein was removed by pre-

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cipitation with *N* HCl at pH 4.6 and 2° C. The resulting whey was filtered, condensed by freezing out a portion of the water, dialyzed exhaustively and then lyophilized.

The lactalbumin, β-lactoglobulin and globulin-proteose fractions used in this study were prepared by the method of Kemp (6). The casein was prepared from skimmilk. It was precipitated with *N* HCl at pH 4.6 and 2° C., washed with water and alcohol and air dried.

Protein solutions were prepared in 0.15*N* NaCl, using a few drops of 5*N* NaOH to aid solution. The solutions then were filtered to remove any undispersed protein.

The semi-micro Kjeldahl method of Menefee *et al.* (11) was used for total nitrogen determinations. The factor 6.35 multiplied by per cent nitrogen gave per cent protein.

For the biuret reagent, 50 g. of sodium citrate (dihydrate), 30 g. of Na_2CO_3 (anhydrous) and 5 g. of $CuSO_4 \cdot 5H_2O$ were dissolved in distilled water and made to 1 l. For use, the above solution was mixed with an equal volume of 0.2*N* NaOH. The mixed reagent is stable for at least 2 wk. and is free of turbidity.

The procedure adopted for the biuret test was as follows: The protein solution was diluted with 0.15*N* NaCl and mixed with an equal volume of biuret reagent to give a final concentration of 0.5 to 1.25 mg. of protein per milliliter. The color was allowed to develop for 30 min. at room temperature and then compared with that of a reagent blank using an Evelyn photoelectric colorimeter with a 540 mμ filter. The Cu-protein complex formed under these conditions was stable for at least 24 hr. The Cu concentration in the test solution is 0.03 per cent. This is somewhat lower than recommended by Mehl (9), but higher concentrations were found to decrease the sensitivity of the test.

The 540 mμ filter was chosen by transmittance determinations of the Cu-protein complex, with milk serum protein, and using a Beckman spectrophotometer, model DU. The chosen wavelength agreed with that recommended for maximum absorption by several investigators (8, 9, 10).

The standard curve (fig. 1) was established by biuret analyses of milk serum protein solutions prepared from several lots of milk. The galvanometer readings obtained were plotted against protein content, as determined by the Kjeldahl method.

RESULTS

Applicability of the standard curve. Lactalbumin, β-lactoglobulin and globulin-proteose fractions were made up in 0.15*N* NaCl solutions. The total nitrogen in these solutions was determined by the Kjeldahl method. Various dilutions of these solutions with 0.15*N* NaCl then were analyzed colorimetrically. All gave biuret tests free of turbidity.

Figure 2 shows the galvanometric readings plotted against concentration of protein, with the standard curve for milk serum protein drawn in for comparison. The values for the various fractions all fall along the standard curve. Thus, the standard curve is applicable to any mixtures of these proteins and can

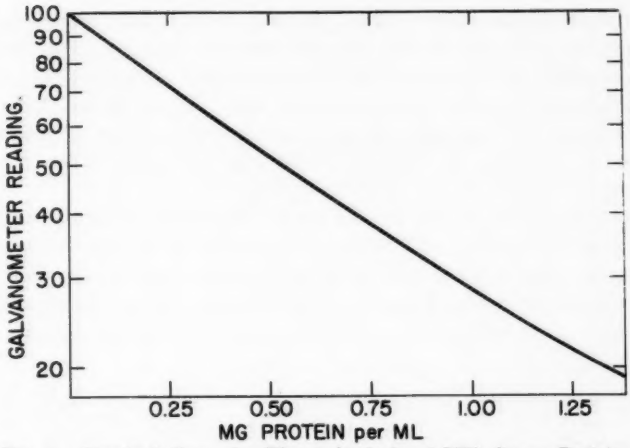

FIG. 1. Standard Curve for Biuret Analysis of Milk Serum Proteins.

be used in studies of milk serum protein fractionation where such mixtures are present.

Casein solutions also were analyzed by the biuret test. The plotted values for casein do not fall on the standard curve; therefore, a separate curve is necessary to analyze this protein. It follows that mixtures of casein and milk serum protein, such as in milk, cannot be analyzed accurately by the biuret test.

Several investigators have studied the response of various proteins to the biuret reagent. Baudet and Giddey (3) found that low values are given by proteins containing an appreciable proportion of non-peptide nitrogen. On the other hand, Hawk *et al.* (5) stated that the biuret reaction was given by all

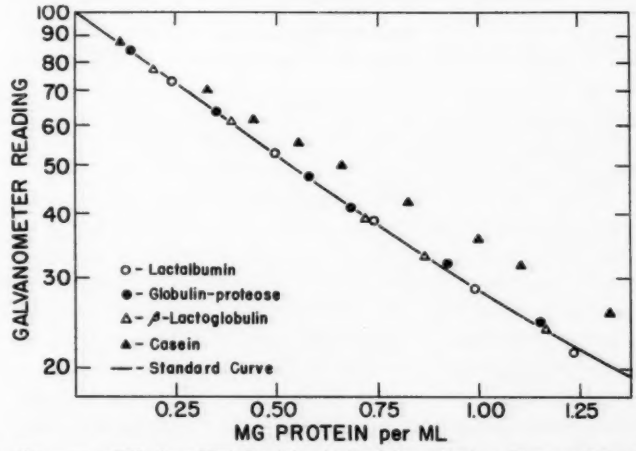

FIG. 2. Response of Various Milk Proteins to the Biuret Test, as Compared to the Standard Curve for Milk Serum Proteins.

substances which contained two amino groups in their molecules, when these were joined either directly or through a single atom of nitrogen or carbon. Histidine and arginine are of this type. In addition, such groups as $-CSNH_2$ $-C(NH)NH_2$ and $-CH_2NH_2$ respond to the test. The latter group is found in lysine. However, the arginine, histidine and lysine contents of casein, β-lactoglobulin and lactalbumin are not widely different (4). Therefore, other factors may account for this variation in color development.

Applicability of the biuret test to whey. The data in figure 3 illustrate a typical trial in which whey and dialyzed whey diluted to various concentrations of protein with 0.15 *N* NaCl were analyzed by the biuret test. The whey was dialyzed overnight in 0.5-in. diameter Visking casing, against running tap water. Total nitrogen and non-protein nitrogen were determined by the Kjeldahl method.

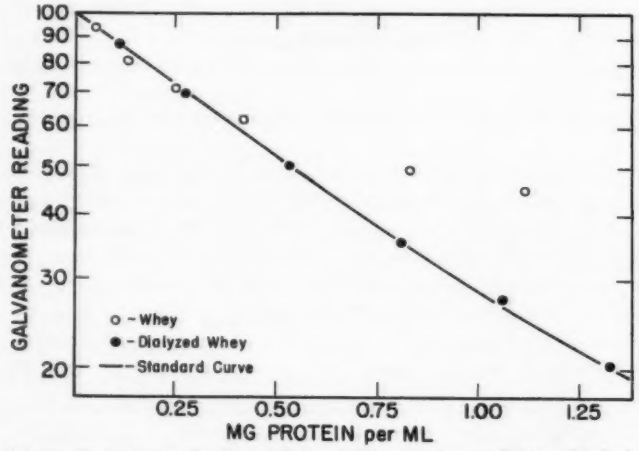

FIG. 3. Response to the Biuret Test of Whey, Before and After Dialysis.

These curves show that the response of the dialyzed whey agreed closely with that for purified milk serum protein, as in the standard curve, whereas the curve for untreated whey deviated widely from the standard curve. Accurate analysis of untreated whey was impossible because lactose reduced the cupric reagent and because the test solutions became turbid when Ca and Cu phosphates precipitated. Cu reduction and clarity of solution were found to depend upon incubation time prior to reading the tests and upon the whey concentration. However, shortening the incubation time did not allow full development of the color complex, whereas diluting the whey sufficiently to eliminate reduction and turbidity also diluted the protein too much for accurate determination.

No interference was noted in any of the trials with dialyzed whey. In addition, the dialysis removed non-protein nitrogenous compounds, thereby obviating correction for this nitrogen fraction.

Applicability to protein fractionation studies. The biuret test cannot be used

in the presence of $(NH_4)_2SO_4$; however NaCl, Na_2SO_4 and ethanol, in concentrations as used in protein fractionation, interfered with the test only slightly. Standard curves were developed for milk serum protein solutions containing known concentrations of these protein precipitants. As an example, a series of curves is shown in figure 4 for Na_2SO_4-protein solutions.

SUMMARY

The biuret reaction was applied to a quantitative colorimetric test for milk serum protein solutions.

The method is simple and rapid, the solutions are free of turbidity and the response is nearly linear. Full development of the Cu-protein color requires about 30 min., after which the color is stable at least 24 hr.

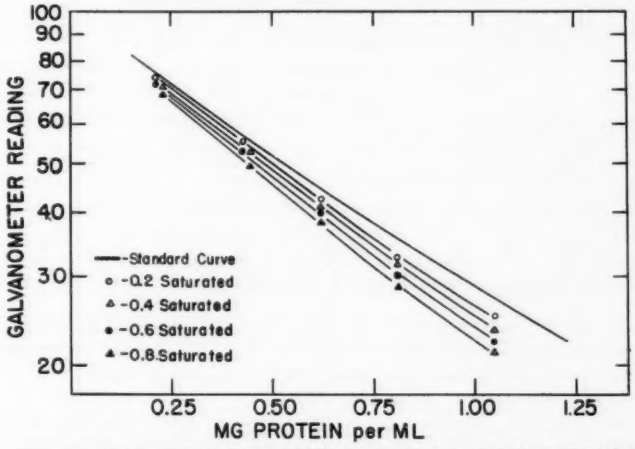

FIG. 4. Response to the Biuret Test of Milk Serum Protein Solutions Containing Various Concentrations of Na_2SO_4. Salt concentrations shown are before addition of biuret reagent.

Lactose and calcium phosphate were found to interfere in direct application of the test to whey. Dialysis of the whey was necessary to obtain normal values.

Concentrations of NaCl, Na_2SO_4 or ethanol as used in protein fractionation interfered with the test. Standard curves were prepared for protein solutions containing known concentrations of these reagents.

The color response of casein is different than that for milk serum protein; therefore, mixtures of these proteins cannot be analyzed by the biuret test.

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GENETIC RELATIONSHIPS IN RESISTANCE TO MASTITIS IN DAIRY CATTLE[1]

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Known individual and species differences in ability to resist infections of certain diseases furnish evidence that genetic differences play a part in conditioning resistance to disease. Plant breeders have been able to develop resistant varieties and strains, but preventive and therapeutic measures largely remain the basis of disease control in economic animal populations. In dairy cattle mastitis presents a disease problem that has not been controlled effectively by either prophylactic or therapeutic measures. The problem has received only limited attention from a genetic standpoint, but the tremendous economic significance of this disease demands attack from every feasible approach.

Several authors have pointed out specific selected instances which indicated that the susceptibility to mastitis manifested by related animals might have a genetic basis (7, 11, 12). Lush (6) has determined the intra-herd heritability of resistance to mastitis to be 0.38 from daughter-dam comparisons involving 494 dams in 27 New Zealand dairy herds.

The results of the present inquiry are presented to furnish additional information with regard to the heritability of resistance to mastitis and thereby permit a clearer appraisal of the practicability of augmenting present preventive and control measures with a program of breeding for mastitis resistance.

EXPERIMENTAL

The data were taken from 11 North Carolina dairy herds which included a total of 1,174 cows. However, 215 animals tested only during their first lactations were not included in the analysis, since there is a marked difference in the incidence of mastitis in first-calf heifers as contrasted to older cows (5). The average incidence of mastitis among the 215 heifers was only 16.7 per cent as compared to 51.1 per cent for the 959 older cows.

Individual quarter samples were collected on a monthly basis for the College herds which included 307 animals. The remaining herds were sampled on a bimonthly basis. All cows in milk were sampled during each visit. Table 1 shows the period during which each of the herds was under observation.

Milk samples were collected using sodium azide to depress the multiplication of possible bacterial contaminants during the 12-hr. incubation period. Following incubation leucocyte and bacterial counts were made using the methods of Prescott and Breed (9) and Baker and Breed (2). During the initial years, the Hotis test also was run on the samples.

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A cow was regarded as susceptible (infected) and given a numerical rating of one if milk from any one of her quarters during the period of sampling showed a leucocyte count of 500,000 or greater per milliliter, accompanied by demonstrable streptococcus or staphylococcous organisms. Bryan (3) concluded that streptococci accounted for 98 per cent of all infectious mastitis; and, although Packer (8) found *Staphylococcus aureus* also to be an important pathogen, staphylococcus and streptococcus organisms were present in 92.3 per cent of the samples in which a positive bacterial diagnosis was obtained. This classification of susceptibility would not include non-specific mastitis, but it was deemed necessary to require the concomitant presence of the bacterial organisms to eliminate the inclusion of cases of high leucocyte count in early or advanced lactation when no pathological udder condition actually existed.

TABLE 1

Summary of data by herd-breed groups

Herd no.	Breed	Year(s) tested	No. cows	No. susceptible	% susceptible
1	G	1944–50	51	28	54.9
1	H	1944–50	40	27	67.5
1	J	1944–50	74	44	59.5
2	H	1943–44	22	9	40.9
3	H	1948	57	22	38.5
4	H	1943–44	46	21	45.7
5	A	1943–49	48	22	45.8
6	G	1947–48	8	1	12.5
6	J	1947–48	27	2	7.4
7	A	1943–50	98	51	52.0
8	J	1945–46, 49	60	12	20.0
9	J	1945–46	23	16	69.6
10	G	1943–46	41	24	58.5
11	J	1943–45	364	211	58.0
Totals			959	490	51.1

Although attacks of mastitis vary widely in their severity, a satisfactory method of rating mastitis infections on a continuous scale is not presently available. These data were of a binomial nature, since the rating was either resistant or susceptible. No transformation of the data was made, however, since the average incidence of mastitis approached 0.5 and was in the region where transformation of the data would add little to the precision of the variance estimates.

RESULTS

The data were analyzed to determine the intra-herd, intra-breed heritability by the daughter-dam regression and the correlation between paternal half-sisters. In the daughter-dam analysis (table 2) when a dam had more than one daughter all of the daughters of the dam were averaged to give a single daughter-dam comparison for the particular dam. The intra-herd, intra-breed regression of 0.07 is not statistically significant. However, the information from the daughter-dam comparison was pooled with the information gained by comparison of groups of paternal half-sisters given in table 3 to determine the fraction of the total

intra-herd, intra-breed variance which could be defined as additively genetic. Twice the daughter-dam regression and four times the paternal-half-sister correlation yielded the estimates of heritability which were pooled. The combined estimate obtained by weighting each of the values from the foregoing analyses by the inverse of their variance is 0.27 ± 0.10.

TABLE 2

Covariance analysis of mastitis rating for dams(x) and daughters(y)

Source of variation	Degrees of freedom	Σx^2	Σxy	Σy^2	Regression y on x
Total	225	52.248	6.539	55.090	.....
Among herd-breed classes	12	7.244	3.394	4.334	.....
Within herd-breed classes	213	45.004	3.145	50.756	0.07 ± 0.07

From table 3 an estimate of the importance of dominance deviations from the additive scheme can be obtained, since the component D in its genetical interpretation is made up of one-quarter of the additively genetic variance plus one-quarter of the variance attributable to dominance deviations (13). The component S represents one-quarter of the additively genetic variance; hence, the expected difference between D and S is one-quarter of the variance attributable to dominance deviations. This gives an estimate of 0.062 for dominance variance in contrast to 0.092 for additively genetic variance from the analysis presented in table 3.

TABLE 3

Analysis of variance of mastitis rating among and within sires

Source of variation	Degrees of freedom	Mean square	Expected value of M. S.	Variance components
Total	958	0.2501		
Herd-breed groups (H-B)	13	1.3801		
Among sires within H-B	295	0.2762	$E^a + 1.021\ D^b + 2.545\ S^c$	0.0231
Among pat/2 sisters	591	0.2195	$E + 1.078\ D$	0.0385
Among full sisters	59	0.1780	E	0.1780

$$\text{Pat/2 Sister Correlation} = \frac{S}{S + D + E} = 0.096 \pm 0.034$$

[a] E is variance among full sisters.
[b] D is additional variance among paternal-half sisters.
[c] S is additional variance among sets of paternal-half sisters.

For five of the herd-breed groups including 283 cows, production records in addition to the mastitis ratings on the individual animals also were available. The 305-day, 2X, mature-equivalent milk and fat yields for the first lactation of each of the cows were used. Choice of the first lactation was made since the low incidence of infection during the first records would leave them reasonably free of direct vitiating influence of mastitis on production.

Using the intra-herd, intra-breed variances and covariances, the phenotypic correlations between production (fat and milk) for the first lactation and mastitis were essentially zero. The correlation between fat and mastitis was 0.006

and that between milk and mastitis was 0.015. Genetic covariances for mastitis and production were determined also from daughter-dam and paternal half-sister comparisons, but interpretable results were not obtained, presumably due to the paucity of available comparisons.

DISCUSSION

The value of 0.27 for the heritability of resistance to mastitis is within sampling agreement with the earlier figure 0.39 obtained by Lush (6). Heritability figures as high as these suggest that some progress in reducing incidence to mastitis might have resulted from simple mass selection. Nevertheless, according to figures reported by Asdell (1), the proportion of cows leaving dairy herds with udder troubles, presumably largely mastitis, has declined only slightly during the past 15 yr. It is true that increased use of milking machines and a multitude of other practices may have obscured the genetic trend. Moreover, most cows do not have attacks of mastitis severe enough to warrant eliminating them from the herd during the first or second lactations. Consequently, a cow has the opportunity to leave one or more offspring prior to the time she is eliminated from the herd because of mastitis. In addition, the intensity of selection apparently is low, since Asdell (1) reports a 2 to 3 per cent annual culling rate for udder troubles and mastitis. As a result, such mass selection among females would seem to be ineffective.

With the present and continued growth of artificial insemination, the progeny testing of bulls for their transmission of genes influencing resistance offers a possible breeding plan to aid materially in reducing the incidence of mastitis. Such a program has its limitations, however, since a satisfactory phenotypic evaluation of the daughters of a sire by present standards must be delayed until the daughters have completed their second or third lactations. Without an early individual test for mastitis, most of the actual selection must be based on pedigree or family information, with the bull's dam and his sisters receiving major emphasis.

This points out the need for more reliable information on methods for evaluating individual phenotypes. Especially needed is a procedure for early appraisal of resistance to enhance the prospects of a successful breeding program. It also should be noted that in rating mastitis on an all-or-none basis, as in the present study, a minimum value of heritability is estimated. Further study appears warranted to develop a measure of resistance on a continuous scale by proper weighting of such things as age of the animal at first attack and severity of the attack, whereby measurable heritability might be increased.

The negligible phenotypic correlation between mastitis rating and production during the first lactation does not support the often expressed belief that mastitis is manifest most strongly in high producers. Possible genotypic and environmental interactions where the relationship might change when cows are provided an opportunity to reach the ultimate of their producing ability, however, also need study. The cows included in this study were not provided such an opportunity, as the average production level of 9,165 lb. of milk and 401 lb. of fat on a 305-day, 2X, mature basis indicates.

In view of the small number of full sisters available, the indication that dominance deviations contribute to the total genetic variance are suggestive only. Evidence for such specific genetic effects or nicking generally has not presented itself in non-inbred material. Seath and Lush (10) did not find evidence of nicking with regard to production in dairy cattle. Likewise, Hazel and Lamoreux (4) found nicking to be only a minor contributor to total genetic variability for sexual maturity and body weight in poultry.

SUMMARY

Data from 11 North Carolina dairy herds involving 956 animals were studied, and an estimate of heritability of resistance to mastitis of 0.27 ± 0.10 was obtained. The data also suggest that dominance deviations contribute to the total genetic variance. No demonstrable phenotypic relationship between mastitis and production was evident from the data. Use in artificial insemination of sires whose dams and sisters demonstrate resistance to mastitis presents the most promising immediate breeding approach.

ACKNOWLEDGMENTS

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FURTHER STUDIES ON THE INFLUENCE OF TOCOPHEROL SUPPLEMENTATION ON THE VITAMIN CONTENT OF THE MILK FAT, STABILITY OF MILK AND MILK AND FAT PRODUCTION

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It has been shown at this Station that feeding of cod liver oil to cows renders milk more sensitive to deterioration resulting in the development of oxidized flavors, and involving ascorbic acid oxidation and that feeding mixed tocopherols along with oil augments the ability of milk to resist these oxidative changes (4, 5, 7, 17). Likewise, more stable milk was produced when barn-cured birdsfoot trefoil hay (*Lotus corniculatus*) of excellent quality was fed to cows as compared with the milk from cows which received poor quality late-cut hay (5, 8, 13). While several factors may be involved (4, 5, 6, 9, 10, 11, 12), it was desirable, nevertheless, to learn the possible value of a tocopherol supplement in maintaining the stability of milk during the winter season when large amounts of poor quality hay are fed, especially since dairy plants experience most trouble with oxidized milk during the winter.

The study was planned also to test more critically the influence of tocopherol supplements upon the fat content of milk, using longer experimental periods than previously reported (17).

EXPERIMENTAL

Eight Holstein cows divided into two equal groups were used in this study. Both groups received a basal ration of late-cut timothy hay (U. S. no. 3 Grade), well-eared corn silage and a commercial concentrate mixture containing 18 per cent protein. Records were kept of the amounts of feeds consumed. Following a preliminary period of 2 wk., the ration of the cows in group 1 was supplemented with 2 g. of mixed tocopherols[1] daily, while group 2 was retained as a control. Period one was of 14-wk. duration and was followed by a second period of 5 wk., during which group 1 was given the basal ration only, and group 2 received the tocopherol supplement. At the end of this period, the tocopherol supplement was discontinued and both groups of cows were transferred to a pasture of mixed birdsfoot trefoil and blue grass.

Milk was weighed and sampled at each milking for the Babcock fat test which was made weekly on composited aliquots. Samples of milk were taken weekly from the morning milking of each cow for studies on tocopherol content and stability of the milk. The milk was pasteurized at 62° C. for 30 min., and the stability of the milk was determined on the basis of its ability to resist the reactions which produce oxidized flavors during 7-day storage at 0 to 5° C. One tenth mg.

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[1] "Myvadry." Distillation Prod., Inc., Rochester, N. Y. ("Distilled tocopherol concentrate on soybean-flour containing 2 per cent of mixed tocopherols.")

of Cu per liter was added to all samples of milk prior to storage at refrigeration temperatures to enhance oxidative changes (4). The milk was protected from light throughout the experimental trial.

A quart of each weekly sample of milk was separated by gravity creaming. The cream was churned and the butter obtained was melted, centrifuged clear, filtered and the fat was analyzed for tocopherol content using Quaife (15) and Quaife and Harris (16) methods.

RESULTS AND DISCUSSION

Tocopherol levels of the milk fat. The average tocopherol levels of the weekly samples of milk are presented in figure 1. The data reveal that during the first 14-wk. period, the tocopherol content of the fat diminished progressively

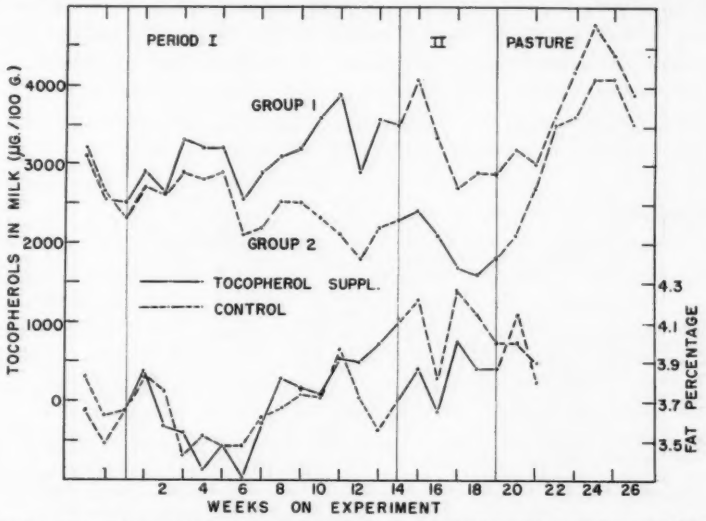

FIG. 1. The average tocopherol content of the milk fat of weekly samples as influenced by standard winter ration, tocopherol supplement and pasture.

when the cows (group 2) were fed the standard ration only, suggesting that the vitamin intake from the basal ration was inadequate to maintain the body reserves of the cow and the amount secreted into the milk. On the basis of an estimated dry matter intake per cow per day and the analysis of the feedstuffs fed to the cow, the animal consumed 1.951 g. tocopherols daily. By comparison, the supplementation of standard ration with mixed tocopherols during the 14-wk. period (group 1) resulted in a consumption of 3.951 g. tocopherols per day, and the vitamin content of the milk fat not only was maintained at much higher levels, but actually showed a slight increase over that for the prefeeding samples of milk fat.

However, when at the end of the 14th week the treatments were changed, and the cows in group 2 were fed mixed tocopherols, the vitamin content of the fat of

both groups of cows declined. At this time the supplement was analyzed and found to contain slightly less than half of its original potency. It is possible that a daily intake of approximately 2.9 g. tocopherols per cow (supplement plus feedstuffs) was not adequate to maintain the vitamin level of the milk fat. However, the ecliptic-like path of tocopherol content of the fat and especially its leveling off toward the end of second period (figure 1), indicate that the physiological response of the cow to the feed consumed, together with the negative effect of steadily deteriorating ration on the assimilation and deposition of tocopherols into the milk fat could not be disregarded. The latter statement is supported also by the data in figure 1, which show that the subsequent feeding of birdsfoot trefoil-blue grass pasture effected a sharp increase in the tocopherol content of the fat when the cow consumed approximately 3.7 g. tocopherols per day.

Fat production. The average fat percentage of the milk is shown in figure 1. Feeding mixed tocopherols over a period as long as 14 wk. had no significant effect on the average fat test. Likewise, the average daily production of 4 per

TABLE 1

Influence of ration on the development of oxidized flavors and the average tocopherol content of the milk fat

Supplement	Period	Oxidized samples		Stable samples	
		No.	Tocopherol content of fat	No.	Tocopherol content of fat
	(*wk.*)		(μ*g./100 g.*)		(μ*g./100 g.*)
		Group I			
Tocopherol supplement	I	14	10	2,493 ± 451	46	3,322 ± 682
Control	II	5	2	2,777 ± 163	18	3,233 ± 743
Pasture	III	7	0	—— ——	28	3,855 ± 843
		Group II			
Control	I	14	52	2,404 ± 404	4	2,835 ± 231
Tocopherol supplement	II	5	20	1,932 ± 420	0	—— ——
Pasture	III	7	3	2,309 ± 242	25	3,509 ± 718

cent fat-corrected milk (1) was not affected (data not shown). These observations are in agreement with those of Phillips *et al.* (14), Gullickson *et al.* (2) and Whiting *et al.* (17), and fail to confirm the report of Harris *et al.* (3).

Oxidized flavors. Summary data showing the number of samples of milk which either underwent or resisted oxidative deterioration during 7-day storage, together with the mean tocopherol content of the fat for each feeding period, are presented in table 1.

Samples of milk which underwent oxidative deterioration averaged low in tocopherol content as compared with those which remained stable. The difference in the average tocopherol content of stable and oxidized samples of milk was statistically highly significant ($P < 0.01$). The data in table 1 and figure 1 reveal, however, that the cows (group 2) which were fed standard ration alone during the first 14-wk. trial, produced milk with fat of comparatively low tocopherol content, and the milk itself showed practically no resistance to oxidized flavors. Supplementation of the ration with mixed tocopherols during the fol-

lowing 5-wk. period, failed not only to counteract the decrease in tocopherol content of the fat, which dropped to its lowest level, but also to improve the resistance of milk to deterioration. By comparison, both the tocopherol content of the milk fat and the resistance of milk to oxidative deterioration were significantly improved (group 1) when the standard ration was supplemented with mixed tocopherols throughout the first 14-wk. trial. Moreover, this beneficial effect of the supplemental feeding extended itself to milk produced on standard ration only during the following 5-wk. period.

When the cows were placed on excellent pasture, the tocopherol content of the fat increased rapidly to high levels, and the unstable milk regained its ability to resist deterioration within 2 to 3 wk.

Whether the large intakes of tocopherols and the physiological response of the cow to the feed consumed are entirely responsible for the results obtained can not be stated at the present time. There is a reason to believe that certain pasture herbage and some roughages fed to the cow during the winter season contain other factors influencing the reactions which produce the oxidized flavors.

These tests show clearly, however, that tocopherol supplementation cannot be depended upon to prevent or correct oxidized flavors in milk under all conditions.

SUMMARY

When Holstein cows were fed a standard winter ration of late-cut timothy hay, corn silage and commercial concentrate mixture, a gradual decrease occurred in the tocopherol content of the milk fat and the milk showed practically no resistance to oxidative deterioration resulting in the development of oxidized flavors.

Supplementation of standard ration with 1 to 2 g. daily of mixed tocopherols at the beginning of the trial appeared to improve both the tocopherol levels of the fat and the ability of milk to resist the reactions which produce the oxidized flavors. No such improvement occurred when the tocopherol feeding was shifted to cows toward the end of winter season.

The data show that the tocopherol supplement cannot be depended upon to maintain the tocopherol content of the fat and to prevent or correct oxidized flavors under all conditions.

Feeding mixed tocopherols over 14-wk. period (1 to 2 g. daily) had no significant effect on the average fat tests.

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CONTENTS OF THE ALIMENTARY TRACT OF CALVES AT BIRTH[1]

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Studies on the size and composition of the whole bovine fetus and of various separated organs and tissues have been reported (5). Only sketchy information, however, has been found in the literature on the quantities and composition of materials found in the alimentary tract of calves at the time of birth. In summarizing work extending back into the seventeenth century, Needham (11) records that various fluids and solids, including hair, had been observed in the alimentary tract of new-born calves. Windle (17) cites similar observations on other mammals. Composition of contents of the intestinal tract of new-born human infants (4, 7, 12, 15, 16) apparently has received somewhat more study than that of calves. Interest has arisen recently in blood-group-specific substances of meconium from infants (3, 14).

The present report is on the quantities and some of the properties of the contents from various parts of the alimentary tract of new-born calves.

PROCEDURE

Normal male dairy calves were used in this study. The calves were slaughtered at birth, and the whole digestive tract was removed immediately. The tract was separated into various sections and the contents were removed. When, in a few instances, this could not be done at once, the organs were placed under refrigeration. In removing materials from the small intestine, considerable care was required to remove all contents without removing some of the tender mucosa lining the tract. It was unavoidable that small amounts of the latter material were included, and also that small amounts of the intestinal contents were not removed. Difficulty was encountered in keeping cecum and intestinal contents separated, since the former usually were more fluid than the latter. By careful handling and tying off sections, mixing was kept at a minimum. The contents of the different sections of the stomach of only two of the calves were collected separately.

The pH of the various materials was determined on the well-mixed samples at room temperature (22 to 25° C.). Nitrogen was determined by the Kjeldahl method. On materials from two of the calves, a non-protein-nitrogen filtrate was prepared by adding water to the sample, homogenizing to a uniform slurry, precipitating protein with trichloroacetic acid and separating the filtrate by a combination of centrifugation and filtration.

The ether extract of contents of various parts of the digestive tracts of seven

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calves was obtained by spreading the sample on fat-free cotton and drying prior to extraction, except that colon contents were dried, ground and extracted in the usual manner. Samples of digestive-tract contents from two other calves, however, were dried by mixing and grinding with anhydrous Na_2SO_4 in a mortar before extraction. Solids and ash contents were obtained in the usual manner (1), and N.F.E. was obtained by difference. Appropriate standard qualitative chemical tests or spectrographic analyses (2) were used for identifications of specific minerals.

RESULTS

In table 1 the data on breeds and weights of new-born calves used in this study are presented. Also, the weights of contents from various parts of the digestive tract are shown on an as-collected and on a dry-matter basis. Weights

TABLE 1

Quantities of materials obtained from various sections of alimentary tracts of newborn calves

Breed	Weight of calf	Total weight of contents				Dry weight of contents			
		Stomach	Small intestine	Cecum	Colon	Stomach	Small intestine	Cecum	Colon
	(*lb.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)
Ayrshire	89	36	159	152	512	0.5	14.8	4.5	144
Guernsey	71	28	55	143	381	0.4	5.3	7.7	100
Guernsey	99	80	177	78	424	1.2	21.8	5.2	111
Jersey	62	173	285	40	450	2.0	23.0	1.3	106
Holstein[a]	102	122	250	40	533	1.7	25.0	1.6	125
Jersey	44	250	230	20	214	3.1	6.2	1.1	64
Guernsey	62	35	145	141	288	0.7	7.3	7.0	78
Ayrshire	84	78[b]	305	73	570	3.0	38.7	3.9	68
Holstein	91	124[b]	222	39	371	1.7[c]	19.9	1.7	123

[a] All contents analyzed as obtained from digestive tract except hair filtered from stomach contents of this calf, due to large quantity and inability to obtain a sample otherwise.

[b] Contents of rumen and reticulum collected separately from contents of omasum and abomasum. The respective weights of the contents from the Ayrshire were 40 and 38 g., and from Holstein, 100 and 24 g.

[c] Rumen and reticulum contents analyzed separately from those from other 2 stomach compartments; weights of contents of rumen and reticulum, 1.2 g. and of omasum and abomasum, 0.5 g.

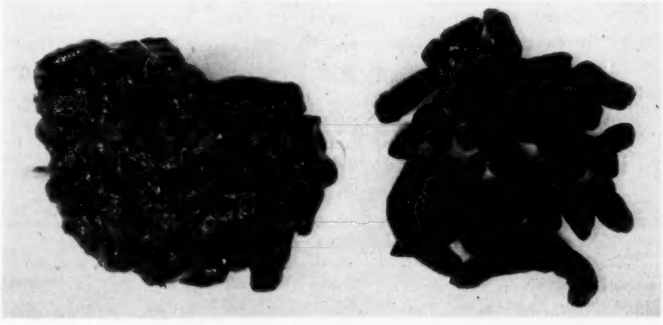
of dry contents from each section of the digestive tract usually correlated roughly with corresponding total weights of contents obtained. However, some marked exceptions were noted. The 84-lb. Ayrshire obviously had defecated and passed considerable meconium before birth, since many small chunks of this material were found in the stomach, principally the rumen. As a result, the colon contents were markedly lower in solids and did not contain the dry fecal pellets usually observed. There was a tendency for the amount of dry matter obtained from the alimentary tract to increase with increasing birth weight of calves.

Hair was observed in the contents from all sections of the digestive tract of all calves, but amounts varied considerably. The stomach contents of the 102-lb. Holstein contained such quantities of long hair, mostly white, that it was impossible to sample. Therefore, the hair was filtered off before analysis. Many of

the hairs were well over 1 in. in length. This hair apparently came from the coat of the fetal calf, and probably was obtained either by shedding into amniotic fluid and subsequent drinking of the fluid by the calf, or by the calf licking itself before birth.

The color and consistency of the samples obtained from different parts of the digestive tract varied. Stomach contents varied from almost colorless and slightly cloudy to light yellow, containing a mucous-like material and, frequently, a few small chunks of meconium and of a curd-like material. Some of the color probably was the result of regurgitation of fluid from the small intestine and/or swallowing of amniotic fluid colored by meconium passed before birth. When collected separately, contents from the first two stomach compartments weighed more than those from the last two (table 1). The rumen and abomasum contained nearly all of the fluids and solids found in the stomach compartments.

Contents from the small intestines of the different calves were fluid to semi-solid and varied from a medium yellow to a brownish yellow color. Material obtained from the lower end of the small intestine was drier and darker in color than that from the upper end.



A B

FIG. 1. Material removed from lower end of colon of a new-born calf (A) and "pellets" separated from this mass (B).

Material from the cecum was darker but less viscous than that representing mixed contents of the small intestine. Cecum contents were a variable mixture of watery, yellowish fluids and brownish, semi-solid materials. On standing, this material often separated slowly into a greenish upper and a yellowish lower layer.

Except for the sample from the 84-lb. Ayrshire, colon contents were viscous, sticky and of a relatively high solids content. The color of colon contents usually was a dark greenish-brown; sometimes, however, yellow material was observed in some of the mass. The last 7 to 10 in. of the colon of most calves usually were packed with a material that might be called fecal pellets, embedded in a sticky viscous mass (fig. 1). The sample from the 89-lb. Ayrshire contained an especially large amount of hair. Hair was found in both the pellets and the viscous material from colons of all calves. The pellets were firm, not easily broken and

could be washed from the intestinal contents with little disintegration. Material in the colons 18 in. or more from the anus was beginning to form into aggregates that finally become the pellets. These aggregates were larger, more compact and drier the lower they were found in the colon. The colon contents did not have the characteristic odor observed on feces of calves receiving food.

TABLE 2

pH of contents from various sections of the alimentary tract of calves at time of birth[a]

Source of material	Av.	Range
Stomach	6.3	4.2–7.2
Rumen + reticulum	6.4	6.3–6.7
Omasum + abomasum	4.4	4.0–4.8
Small intestine	6.6	6.2–6.7
Cecum	6.7	6.3–7.3
Colon	5.7	5.3–5.9

[a] Data from nine calves of 4 dairy breeds, except data on pH of total stomach contents were from 7 calves, and of stomach compartments, 2 calves.

The pH of contents from the digestive tracts of calves is shown in table 2. A wide range of values was obtained on stomach contents. The relative amounts of acid secreted in the abomasum, regurgitation from the small intestine and amniotic fluid swallowed immediately prior to birth probably determined the pH of stomach contents. Less variation was found in the pH of contents of the small intestines of calves than in that of contents from any other part of the digestive tract. The pH of cecum contents was similar to, but averaged slightly more basic than that from the small intestine. The contents of the colon were

TABLE 3

Proximate analysis of contents from various sections of the alimentary tracts of calves at time of birth

Source of material	Total solids	Crude protein	Ether extract	Ash	N.F.E.[a]
	(%)	(%)	(%)	(%)	(%)
Stomach[b]	1.8	0.88	0.15	0.85	
	1.2–3.8[c]	0.25–2.0	0.05–0.79	0.74–0.91	—
Small intestine	8.7	5.4	0.98	0.99	1.7
	5.1–12.7	1.3–8.7	0.17–2.2	0.66–1.1	0.28–5.8
Cecum	4.7	2.1	0.26	0.82	1.6
	2.9–6.6	0.97–3.3	0.06–0.74	0.70–0.95	0.80–3.3
Colon	25.2	11.3	3.6	1.1	9.0
	12.0–33.1	6.4–15.2	2.3–10.5	0.95–1.2	4.0–12.0

Samples obtained from 9 calves.

[a] Crude fiber data only on colon contents of 3 calves (av., 0.19; range, 0.11 to 0.24).

[b] All materials analyzed as obtained, except hair filtered from stomach contents of one calf, since it was impossible to sample material otherwise.

[c] Range.

more acidic than that from any other section of the alimentary tract except the abomasum.

Proximate analyses of the materials from the alimentary tract are given in table 3. It is recognized that the usual proximate analysis is not entirely adequate for investigations of this type. The object, however, was to show results

according to the scheme commonly employed in nutrition studies with farm animals. Average N.F.E. of stomach contents would be negative if calculated. This probably resulted from presence of non-protein nitrogen and from experimental errors that are difficult to overcome when working with samples of a low solids content.

In samples from the stomach, small intestine and colon from the 84-lb. Ayrshire, approximately one-third of the nitrogen was of non-protein origin. Most of the nitrogen of the abomasum contents of the 91-lb. Holstein was of non-protein origin, but only about one-sixth of nitrogen from the colon was non-protein. About one-third of the nitrogen of the rumen, the small intestine and the cecum contents of this calf was non-protein. Samples from other calves were analyzed only for total nitrogen. When the quantities of non-protein nitrogen are subtracted, the protein content of the samples is lowered (about one-third in most cases) and N.F.E. is increased proportionally. On a dry matter basis, the composition of material from the colon was similar to that of a pooled sample of meconium from human infants (14).

TABLE 4

Substances identified in the ash of the contents from the intestines of calves at time of birth

Contents of small intestine[a]													
Ca		Na		Mg		K		Al[b]		Fe		Cu	
	Zn		Mo		Mn		Co		P		Ni[b]		Cl	SO_4
Contents of Colon[c]													
Ca		Na		Mg		K		Al[b]		Fe		Cu	
	Zn		Mo[b]		Mn		Co		P		Ni[b]		Cl	
		Si(?)		V(?)		SO_4		CO_3						

[a] Composite from 2 calves.
[b] Trace only.
[c] Composite from 4 calves.

Substances identified in the ash of small intestine and of colon contents are presented in table 4. The two lists are the same, except that carbonate was not identified in the contents from the small intestine, and there was some question on the tests for silicon and vanadium. Since tests for all elements were not made in this investigation, additional unidentified substances also may be present in the ash.

DISCUSSION

Inspection of the foregoing data leaves little doubt that there is considerable activity of the digestive tract before birth of the calf. This is in agreement with various studies of fetuses of other mammalian species (4, 17).

The large concentration of hair (presumably entering the alimentary tract by the way of the mouth) in the lower colon as compared to that of the stomach or of the small intestine contents indicates that considerable material has passed through the digestive tract and that part of it has been reabsorbed. Although the dry matter of the contents of the colon was three times that of the contents of the small intestine, the ash concentrations of the contents of the small intestine and of the colon were similar. Thus, some of the minerals apparently had been reabsorbed along with water while passing through the colon.

Acid appeared to have been secreted in the stomach, and the small intestine contents were basic prior to the initial feeding.

The substances identified in the ash of intestinal contents include 12 of the minerals recognized as essential in animal nutrition (9), plus others generally not regarded as essential. Possible sources of the substances found in the ash are digestive secretions (pancreatic juice, bile, etc.), desquamated epithelial lining of the digestive tract, amniotic fluid (together with hair, desquamated tissue, etc., found in it) and secretion into the intestine of the fetus. Hair indicates the amniotic origin; bile pigments and pH differences, the digestive secretions; and mucous and cellular debris, the material from the intestinal walls. None of the data obtained specifically points to exchange of substances through walls of the intestine as a source of substances in the digestive tract, but experiments with segregated intestinal loops of older animals suggest this possibility (8).

The question arises whether the fetus receives nourishment from substances passing through the alimentary tract. An interesting and ancient literature exists on this subject (11). Amniotic fluid contains 1 to 4 per cent total solids (10, 13), which includes non-protein nitrogen, protein, various salts and a small amount of reducing substance which was not identified (13). As the calf swallows this material, the nutrients contained are potentially available to the calf. The excess water and perhaps other substances absorbed from the amniotic fluid are given up through the placenta, either by exchange with fetal circulation or by kidney secretions into the allantois.

The solid particles observed in the stomach included hair from which little, if any, nutrient would be obtained by the calf. Small bits of meconium usually are found in the stomach. This, likewise, probably provides little, if any, nutrient, since it principally represents body wastes. At birth the calf is covered with vernix caseosa. Small amounts of material that apparently were derived from this source sometimes were observed in the stomach. This possibly is a source of some nutrient, but probably is relatively unimportant because of the small quantities involved. Processes whereby materials, such as salts, are secreted into the intestinal tract and later reabsorbed possibly play an important part in conserving these respective nutrients in the body of the fetus.

Presence of nutrients in the intestinal tract will not insure digestion and absorption. However, digestive enzymes have been reported in intestinal contents of fetuses of several species (4, 15, 17), and food introduced into the stomachs of fetuses has undergone digestion (6). Furthermore, it has been shown the stomachs of human infants have a well developed capacity to function at birth (4).

SUMMARY

A study was made of the quantities, composition and some of the properties of materials from various sections of the alimentary tract of newborn calves. Data are presented on total weights of contents obtained, on dry weights of contents, on pH and on proximate composition of materials from the stomach, small intestine, cecum and colon. Substances identified in the ash of contents of both the small and the large intestines include 12 of the minerals regarded as essential in animal

nutrition. The possible relation of the alimentary contents of the new-born calf to its prenatal nutrition is discussed.

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THE EFFECT OF AUREOMYCIN SUPPLEMENTATION ON GROWTH AND FEED UTILIZATION OF YOUNG DAIRY CALVES[1, 2]

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Although the growth-stimulating effects of the oral administration of vitamin B_{12} and certain antibiotics have been widely demonstrated with poultry and swine, less information is available relative to the response of ruminants to these substances. Rusoff and Haq (16) and Williams and Knodt (20) observed no apparent beneficial effects when vitamin B_{12} (in APF supplements) was fed to young dairy calves. When crystalline aureomycin hydrochloride (13, 15) or a feed supplement containing aureomycin (2, 3, 9, 10, 14, 19) was added to the diets of calves, however, a growth stimulation and a lowering of the incidence of diarrhea were observed. In contrast to the favorable responses of calves to aureomycin supplementation, Colby *et al.* (5, 6) found that aureomycin feeding depressed growth and reduced the diameter and length of wool fibers in lambs. Similar, although less severe, effects resulted from the oral administration of penicillin and streptomycin (7). Moreover, Bell *et al.* (4) observed diarrhea, anorexia and a reduction in the digestibility of dry matter and crude fiber by steers fed aureomycin.

The present investigation was undertaken to study the effect of aureomycin supplementation on certain physiological responses of calves fed various "practical" and restricted diets.

EXPERIMENTAL

The experimental work to be reported herein was conducted in two phases. The first (experiment I) was initiated to ascertain the effects of aureomycin supplementation on young dairy calves fed and managed under various "practical" regimes. The second (experiment II) was designed to study the effect of aureomycin upon the digestibility and efficiency of utilization of feed nutrients.

Experiment I.

Procedure. The experimental subjects, Ayrshire, Guernsey, Holstein and Jersey calves from the Iowa State College dairy herd, were allowed to remain with their respective dams for 3 days following birth. Subsequently, they were transferred to individual pens, divided into four comparable groups and fed the various experimental diets over a period of 16 wk. All calves received medium quality alfalfa hay *ad libitum* and a concentrate mixture, limited to a maximum of 4 lb. daily per calf, consisting of 40 per cent ground corn, 30 per cent crushed

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TABLE 1

Effect of type of milk and of aureomycin supplementation on weight gain and feed utilization of dairy calves (Expt. I)

Dietary group	Calf Br.	Calf Sex	Initial body wt.	TDN[a] consumption Milk	Cone.[b]	Hay[c]	Total	Body wt. gain	TDN/ lb. gain
			(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
I (Whole milk)	J	M	60	72	174	41	287	154	1.9
	J	F	55	66	159	28	253	124	2.0
	G	F	54	58	143	40	241	96	2.5
	H	M	95	100	169	84	353	192	1.8
	H	F	98	100	236	69	405	178	2.3
	A	F	68	81	149	66	296	139	2.1
	H	M	110	115	262	133	510	178	2.9
	J	F	57	61	115	41	217	64	3.4
	G	M	78	74	157	51	282	102	2.8
		Av.	75	81	174	61	316	136	2.3
II (Whole milk + aureomycin[d])	J	M	64	67	168	73	308	161	1.9
	J	F	60	75	193	29	297	137	2.2
	G	F	70	82	193	51	326	148	2.2
	H	M	95	103	190	106	399	201	2.0
	H	F	91	95	233	94	422	191	2.2
	A	F	64	81	193	91	365	178	2.1
	H	M	84	95	245	136	476	182	2.6
	J	F	41	57	156	41	254	130	2.0
	G	M	63	73	200	65	338	172	2.0
		Av.	70	81	197	76	354	167	2.1
III (Reconstituted skimmilk)	J	M	53	71	100	36	207	98	2.1
	J	F	55	68	169	70	307	142	2.2
	G	F	59	57	68	50	175	69	2.5
	H	M	90	87	166	99	352	169	2.1
	H	F	90	93	171	78	342	136	2.5
	A	M	86	84	206	85	375	167	2.3
	H	F	94	100	169	93	362	144	2.5
	J	M	65	72	132	45	249	114	2.2
		Av.	74	79	148	70	296	130	2.3
IV (Reconstituted skimmilk + aureomycin[d])	J	M	66	87	151	42	280	178	1.6
	J	F	63	71	159	51	281	140	2.0
	G	F	71	82	185	77	344	158	2.2
	H	M	81	96	148	99	343	195	1.8
	H	F	88	97	168	120	385	164	2.4
	A	M	83	97	173	86	356	214	1.7
	H	F	92	104	200	118	422	186	2.3
	J	M	71	88	164	69	321	164	2.0
		Av.	77	90	169	84	342	175	2.0

[a] TDN values used in calculations were: 16% whole milk, 84% non-fat dry milk solids, 77% concentrate and 50% hay.

[b] Concentrate mixture limited to maximum of 4 lb./calf daily.

[c] Fed *ad lib.*

[d] 80 mg./calf daily fed in the milk via nipple pail.

oats, 28 per cent soybean oil meal, 1 per cent steamed bone meal and 1 per cent salt.

To the calves in groups I and II (table 1), whole milk (average fat content, 3.45 per cent) was fed at an initial rate of 10 lb. per 100 lb. body weight daily with a weekly reduction after the first 2 wk. of 1 lb. of milk daily per 100 lb. body

weight. A minimum level of 1 lb. of milk per feeding was maintained to the end of the experiment to facilitate administration of the aureomycin.

The skimmilk fed to the calves in groups III and IV (table 1) was reconstituted so as to be approximately equal in caloric content to the whole milk. The concentration of the skimmilk was increased gradually from 10 per cent solids (1 part spray-process non-fat dry milk solids[4]: 9 parts water) initially to 20 per cent solids (ratio, 1: 4) on the ninth day of the experiment. The skimmilk was fed at the same rate as the whole milk. The daily allowance of milk (whole or skim) for each calf was divided into two equal feedings and was fed via nipple pail. During the initial 28 days of the experiment, each calf receiving the reconstituted skimmilk was fed a daily supplement, in capsules, of 15,000 and 3,000 USP units, respectively, of vitamins A and D.

Crystalline aureomycin hydrochloride, at the rate of 80 mg. per calf daily (40 mg. per feeding), was fed in the milk to calves in groups II and IV. To facilitate feeding, the aureomycin was mixed with non-fat dry milk solids in a ratio of 1: 100.

Whenever evidence of diarrhea was detected, the amount of milk fed was reduced 50 per cent. This level was maintained until the scouring subsided, at which time the intake was increased gradually to the normal level. In severe cases of diarrhea proprietary compounds[5] were administered.

Criteria of the responses of the calves to the various dietaries were health, body weight changes, and milk, hay and concentrate consumption. The calves were examined twice daily to detect abnormalities, particularly diarrhea, and were weighed at weekly intervals.

Results. Data relative to the group distribution of experimental animals, TDN consumption and total weight gains are presented in table 1, and the mean weekly body weight changes for each group of calves are shown in figure 1. The weight gains of calves fed skimmilk were comparable to those of calves fed whole milk, whereas an increase in rate of gain resulted from aureomycin supplementation. The aureomycin-fed calves exhibited an accelerated growth rate early in the experimental period, the differences between the weight gains of calves in Groups II and IV (aureomycin) and those of calves in Groups I and III (no aureomycin) becoming increasingly greater as the experiment progressed. An analysis of variance of the weekly weight data (using only the first eight calves in each group) indicated differences between the aureomycin-fed and the non-supplemented animals that were significant statistically at the $P = 0.05$ level. Since the sex distribution among the eight-calf groups as employed above was not constant, a further analysis of the data was undertaken, employing only the first five calves in each group, where breed and sex distributions were constant. The difference in total mean weight gains between aureomycin-supplemented calves and non-supplemented calves was significant statistically at the $P = 0.01$ level and, as in the analysis of weekly weights in which

[4] Purchased from the Des Moines Cooperative Creamery, Des Moines, Ia. Composition (by analysis): 35.5% protein, 0.2% ether extract, 52.8% nitrogen-free extract, 7.6% ash.

[5] "Calf Scour Compound" produced by Arnold Laboratories, New Castle, Ind. and "Mulsed" produced by Pitman-Moore Co., Indianapolis, Ind.

eight calves per group were included, no significant difference due to type of milk was indicated. Moreover, there was no apparent treatment (aureomycin vs. no aureomycin) × type of milk (whole vs. skim) interaction. Greater variation in total weight gains occurred among the non-supplemented calves than among the aureomycin-fed animals, as indicated by standard deviations of 38 and 24 lb. respectively.

Further analysis of the weight gains of calves in the various dietary groups indicated a breed difference in the response to aureomycin supplementation. The Ayrshire, Guernsey and Jersey calves of the antibiotic-supplemented groups

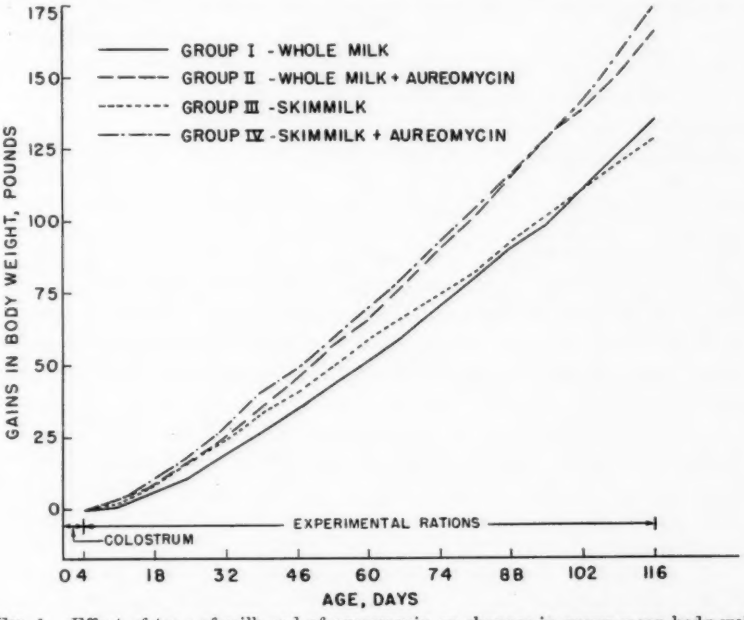

FIG. 1. Effect of type of milk and of aureomycin on changes in group mean body weights (Expt. I).

exhibited average weight gains 41 per cent greater than the gains of the respective non-supplemented animals, whereas the supplemented Holstein calves showed weight gains only 12 per cent greater than those Holsteins not fed the antibiotic. Conversely, no breed × type of milk interactions were apparent.

The data presented in table 1 suggest that aureomycin supplementation enhanced appetite. No major differences were apparent in milk consumption because intake was limited. The aureomycin-fed calves consumed 15 per cent more concentrates than the non-supplemented animals, a difference manifested largely during the terminal 8 wk. (table 2). Full expression of appetite for concentrate was precluded, since each calf was limited to a daily maximum intake of 4 lb. In hay consumption, where feeding was *ad libitum*, the aureomycin-supplemented calves exceeded the non-supplemented animals by 21 per cent. The ratio of cal-

culated total TDN consumption (12) to body weight gain (table 1) indicated a more efficient feed utilization by the aureomycin-supplemented than by the non-supplemented calves.

The frequency of scouring (table 3) was greater during the first 4 wk. of the experimental period for the calves fed skimmilk (groups III and IV) than for those receiving whole milk (groups I and II). Moreover, incidence of diarrhea was somewhat lower among the aureomycin-fed calves (groups II and IV) than

TABLE 2

Mean concentrate and hay consumption of calves in the various dietary groups (Expt. I)

Dietary group	Concentrates, 4-wk. intervals				Hay, 4-wk. intervals			
	1–4	5–8	9–12	13–16	1–4	5–8	9–12	13–16
	(*lb.*)				(*lb.*)			
I (Whole Milk)	15	42	75	93	5	17	39	62
II (Whole milk + aureomycin)	18	50	85	103	7	20	42	83
III (Skimmilk)	11	36	63	82	4	17	43	75
IV (Skimmilk + aureomycin)	8	34	75	105	6	20	48	92

among the non-supplemented calves (groups I and III). Since the frequency of scouring was relatively low, however, especially in the whole milk groups, the effect of aureomycin feeding on diarrhea could not be evaluated accurately. Furthermore, the laxation resulting from high intakes of milk solids-not-fat apparently cannot be eliminated by aureomycin feeding at the level employed in this experiment.

TABLE 3

Mean incidence of diarrhea of calves in the various dietary groups (Expt. I)

Dietary group	Weeks				
	1–4, incl.	5–8, incl.	9–12, incl.	13–16, incl.	Total
	No. times diarrhea observed[a]				
I (Whole milk)	2.6	2.1	0.0	0.4	5.1
II (Whole milk + aureomycin)	1.7	0.4	0.3	0.0	2.4
III (Skimmilk)	11.9	2.4	2.9	0.0	17.1
IV (Skimmilk + aureomycin)	12.4	0.5	0.0	0.0	12.9

[a] Group means. Observations made twice daily (a total of 224 for each calf).

The calves fed aureomycin had smoother haircoats and were more thrifty than non-supplemented calves, a difference which was clearly evident among calves in all breeds but which was less pronounced among the Holsteins than among calves of the other breeds.

Experiment II.

Procedure. The experimental animals, ten Holstein males from the Iowa State College dairy herd, were allowed to remain with their respective dams for 3 days following birth. Subsequently, they were transferred to individual pens, muzzled and allotted randomly to two groups of five calves each (groups V and

VI). All calves were restricted during the initial 8 wk. of the 16-wk. experimental period to a diet of reconstituted skimmilk (non-fat dry milk solids plus water). As in experiment I, the concentration of the non-fat dry milk solids in the milk was increased gradually until a level of 20 per cent was reached after 9 days of the experiment had elapsed. Milk feeding was at the daily rate of 10 lb. per 100 lb. body weight, except that a maximum level of 6 lb. per feeding was established in an attempt to prevent excessive diarrhea.

During the terminal 8-wk. period, the calves were fed hay and a concentrate mixture as in experiment I, and the daily skimmilk allowance was reduced 2 lb. each week until 1 lb. per feeding was reached, a level which was maintained for the remainder of the experiment.

Each calf in group VI received daily 80 mg. of aureomycin hydrochloride in the milk. During the first 10 wk. of the experiment, each calf received daily via capsule 15,000 USP units of vitamin A and 3,000 USP units of vitamin D. When evidence of digestive disturbance was detected the therapy was the same as that employed in experiment I.

The criteria employed for evaluation of responses in experiment I also were employed in experiment II. In addition, three successive 24-hr. samples of urine and of feces were obtained from each calf at 2, 5 and 8 wk. of age. The urine samples were collected under toluene and were analyzed for reducing sugars, employing a modification of the Somogyi method (17), and for total nitrogen by the Kjeldahl method (8).

An aliquot of each 24-hr. feces sample was analyzed for nitrogen by the Kjeldahl method and for dry matter, ash and ether extract employing the methods described by the Association of Official Agricultural Chemists (1).

A small sample of feces, obtained by stimulation of defecation, was collected three times daily from each calf during the fecal collection period and was frozen soon thereafter. Prior to analysis the three samples, collected each 24-hr. period from each calf, were thawed and mixed thoroughly. An aliquot was mixed with distilled water and was analyzed for reducing sugar by a modification of the method of Somogyi (17).

During 1 day of each collection period and again at approximately 12 wk. of age, venous blood samples were taken from each calf prior to feeding and at 2, 4, 6, 8 and 10 hr. postprandially. The amount of skimmilk offered at the feeding prior to the sampling at 12 wk. was increased to 6 lb. for each calf. The anticoagulant employed in the blood samples was a mixture of potassium oxalate and sodium fluoride, the latter also serving to prevent glycolysis (18). The colorimetric method of Somogyi (17) was employed to estimate the reducing sugar levels in the blood samples.

Results. In contrast to the growth stimulatory effect of aureomycin supplementation observed in experiment I, calves fed the antibiotic in experiment II exhibited weight gains similar to the controls during the 8-wk. restricted dietary (table 4). Moreover, no marked differences in incidence of diarrhea or in general appearance were observed between the two groups in the initial phase of the latter experiment. During the terminal phase, however, when hay and a con-

centrate mixture also were fed, the antibiotic-supplemented calves (group VI) exhibited greater weight gains and more efficient feed utilization than the non-supplemented calves (group V), differences which were manifest largely during the last 2 wk. of this period.

TABLE 4

Mean weight gains and TDN consumption of calves on restricted diets (Expt. II)

Dietary group	No. of calves[a]	Initial wt.	1–8 wk., incl.			9–16 wk., incl.					
			TDN[b] intake	Wt. gain	TDN/lb. gain	TDN[b] intake				Wt. gain	TDN/lb. gain
						Skim-milk	Hay	Conc.	Total		
		(*lb.*)				(*lb.*)					
V (Skimmilk[c])	5	94	94	57	1.6	43	42	116	201	80	2.5
VI (Skimmilk[c] + aureomycin[d])	5	86	90	57	1.6	42	29	114	185	94	2.0

[a] All calves were Holstein males.
[b] TDN values used in calculations were: 84% non-fat dry milk solids, 77% concentrate, and 50% hay.
[c] Calves restricted to reconstituted skimmilk for 8 wk., after which hay and a concentrate mixture also were fed.
[d] 80 mg. aureomycin hydrochloride daily in the milk via nipple pail.

Table 5 presents the nutrient "utilization" data for calves in experiment II. There were no great differences between the aureomycin-supplemented and the non-supplemented groups in carbohydrate (reducing sugars) and nitrogen values, although utilization was slightly higher in most instances for calves fed

TABLE 5

Nutrient utilization by dairy calves on restricted diets (Expt. II)

Dietary group[a]	Age, days[b]	Carbohydrate[c] (reducing sugars)	Nitrogen[c]	Dry matter[d]	Ash[d]
		(%)	(%)	(%)	(%)
V (Skimmilk)	16–18	98.2	50.2	96.8	66.4
	36–38	98.6	35.7	95.2	54.4
	58–60	98.3	41.4	94.1	43.3
VI (Skimmilk plus aureomycin)	16–18	99.4	51.9	97.4	72.6
	36–38	98.7	39.0	95.4	57.9
	58–60	98.6	38.2	96.3	65.2

[a] 5 Holstein male calves/ group.
[b] 3-d. collection: each day's sample analyzed separately and average values for all calves presented.

[c] $\% = \frac{\text{Intake} - (\text{fecal} + \text{urinary excretions})}{\text{Intake}} \times 100$

[d] $\% = \frac{\text{Intake} - \text{fecal excretion}}{\text{Intake}} \times 100$

aureomycin. Approximately 98 and 91 per cent of the total carbohydrate and and nitrogen excretions, respectively, were via the urine. Since the lipid content of the diet was low (average, 1.2 g. per calf daily), the excretion of ether-extractable materials in the feces also was small (average, 1.3 g. per calf daily).

Ash utilization (coefficient of digestibility) was higher among the supplemented calves but the difference was not significant at the P = 0.05 level.

Age of the calves had no appreciable effect upon apparent carbohydrate utilization. Retention of nitrogen and digestibility of ash, however, decreased sharply as age increased. This difference may have been due in part, however, to a higher incidence of scouring during the last two fecal collection periods (36 to 38 and 58 to 60 days). Analysis of the fecal excretion data for these latter two periods revealed the following increases during diarrhea (as compared to values for collections made when feces consistency was normal): Feces, 156 per cent; carbohydrate (reducing sugars, 149 per cent; nitrogen, 85 per cent; ash,

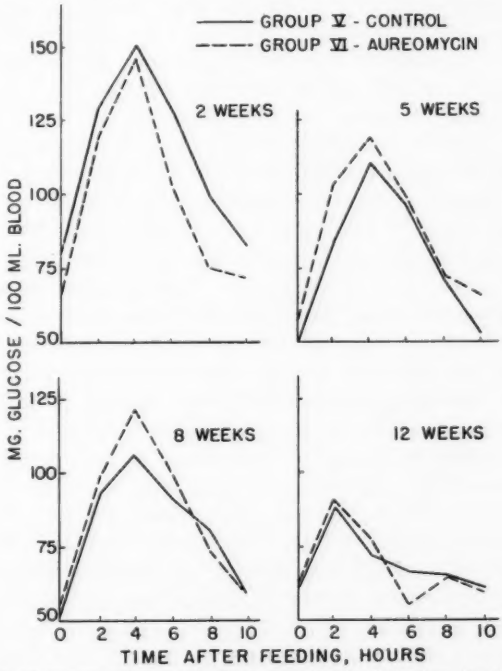

FIG. 2. Effect of aureomycin on blood glucose levels following feeding of skimmilk to dairy calves.

71 per cent; and ether extract, 130 per cent. No appreciable changes in quantity of reducing sugars and nitrogen excreted in the urine occurred during scouring.

Although the mean blood reducing sugar values following feeding (fig. 2) showed greater increases for calves fed aureomycin, the differences were not great. Maximum values were reached at 4 hr., except in the 12-wk.-old calves in which maxima were attained earlier.

DISCUSSION

In the first phase of the present investigation (experiment I) the growth rate of young dairy calves was enhanced by oral administration of aureomycin.

Although unthrifty calves may show a greater response than vigorous calves to antibiotic feeding, as evidenced by the smaller variability in weight gains among the aureomycin-supplemented calves, the data indicate that growth of thrifty calves also is improved, but perhaps to a lesser degree. The observed greater feed intake and more efficient utilization of feed by aureomycin-supplemented calves, which are in accord with observations of Loosli *et al.* (10), suggest that improved appetite is a major factor in the growth-stimulating effect. The cause of the increased appetite is obscure but may be associated with improved health and greater vigor. Although the incidence of diarrhea was slightly lower among aureomycin-fed calves, the comparatively infrequent occurrence of this disorder in all groups indicates that diminished scouring is not the sole cause of the improved growth resulting from feeding of the antibiotic. Moreover, whether the improved general appearance and condition of haircoat readily apparent among aureomycin-fed calves was due to increased feed intake and reduced scouring or to other factors is not clear.

Calves of the Ayrshire, Guernsey and Jersey breeds showed greater responses to antibiotic supplementation than did Holsteins, which is in accord with the observations of Rusoff *et al.* (15) who reported that aureomycin enhanced growth to a greater extent in Jerseys than in Holsteins. The reason for this breed difference may not become apparent until the mode of action of antibiotics is more clearly understood. It seems that further investigation is necessary, however, to determine whether the breed differences observed are characteristic generally or whether they are peculiar to the herds involved.

The results of experiment II indicate that calves fed a restricted diet of the type employed in this study apparently do not respond to aureomycin supplementation in a manner similar to that observed in experiment I. Although efficiency of feed nutrient utilization in most instances was slightly greater for the supplemented calves, the differences were within the range of experimental error. Since the body weight changes of the aureomycin-fed calves were similar to those of the unsupplemented calves during the restricted dietary, it seems possible that full expression of appetite may be necessary for maximum growth response to antibiotics. The possibility exists, however, that the response of calves to aureomycin supplementation might be enhanced by inclusion of hay and/or a concentrate mixture in the diet, even though total dietary intake is restricted and expression of appetite suppressed. In experiment II the aureomycin-fed calves did not consume more feed than the unsupplemented animals but this may have been due to the restricted nature of the diets during the initial phase, to subsequent adjustments to hay and concentrate feeding and to the small number of animals involved.

McCandless and Dye (11) observed that the young of four ruminant species (cattle, sheep, goats and addax antelope) exhibited higher glucose levels than did the adult animals of the same species. The differences were attributed to dietary changes, the young consuming large quantities of milk and the adults consuming chiefly feeds that must undergo changes in the functional rumen. The blood glucose levels shown in figure 2 indicate that the type of diet is not

the determining factor since the mean values at 2 wk. of age were considerably higher than those at 5 and at 8 wk. of age, even though composition of the diet was constant.

A comparison of the weight gains of skimmilk-fed calves in experiment I with those of the whole milk-fed calves indicates that replacement of a large portion of the dietary fat with milk solids-not-fat does not alter appreciably the rate of growth. Since skimmilk contains a small amount of fat and since hay and concentrates were fed at an early age, however, the dietary inessentiality of fat was not established. Moreover, the failure of aureomycin feeding to markedly reduce scouring among skimmilk-fed calves indicates that administration of this antibiotic is not an adequate control for laxation resulting from ingestion of large amounts of milk solids-not-fat.

SUMMARY

In experiment I, 4-day-old dairy calves were divided into four comparable groups and subsequently were fed hay, a concentrate mixture and a limited amount of either whole milk (groups I and II) or reconstituted skimmilk (groups III and IV) for a 16-wk. period. The skimmilk was reconstituted so as to be approximately equivalent in caloric content to the whole milk. Each calf in groups II and IV was fed a daily supplement of 80 mg. aureomycin hydrochloride in the milk via nipple pail.

The aureomycin-fed calves exhibited weight gains that were significantly greater statistically than those of the non-supplemented animals. Conversely, type of milk had no significant effect on rate of gain. The supplemented calves consumed more feed and utilized the feed more efficiently (less TDN per pound weight gain) than the unsupplemented animals. Although incidence of diarrhea was lower among aureomycin-fed calves, the improved growth could not be attributed solely to this factor. Condition of haircoat of the supplemented animals was superior.

In experiment II, two groups of calves (groups V and VI) were restricted to skimmilk diets for an 8-wk. period beginning at 4 days of age and during a subsequent 8-wk. period were fed skimmilk, hay and a concentrate mixture. Calves in group VI received aureomycin at the same rate as those in groups II and IV.

Periodic urine, feces and blood collections and analyses during the restricted dietary period indicated slightly greater efficiency of "utilization" of the various nutrients (carbohydrates, nitrogen, ash and ether extract) by aureomycin-fed calves than by non-supplemented animals but the differences were not significant statistically. Moreover, there were no differences between the two groups in weight gains during the period when the restricted diet was fed.

Blood glucose values for the calves on the restricted dietary regime declined sharply during the period between 2 and 5 wk. of age, despite a qualitative constancy of the diet.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Shirley Davis for technical assistance, to P. G. Homeyer and J. E. Legates for assistance in statistical interpretation of the data, and to Lederle Lab., Inc., Pearl River, N. Y., for the aureomycin.

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EFFECT OF INCIPIENT ENZYMATIC PROTEOLYSIS ON THE VISCOSITY AND HEAT STABILITY OF EVAPORATED MILK[1]

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The physical characteristics of evaporated milk which contribute to its quality are viscosity, grain and color. Viscosity is the most important single factor in determining the keeping qualities of evaporated milk in storage from the standpoint of fat and protein separation. Low viscosity accelerates the rate of fat separation in storage (2, 23, 24). The low viscosity of milk sterilized at high temperature-short time (HTST) deters processors from adopting this method of sterilization. Milk sterilized in this manner is of superior quality with respect to brown discoloration and cooked flavor (22, 23), but on account of its "thin" body a rapid fat separation takes place, limiting the storage life of the milk to a few months (15, 18, 23).

Deysher *et al.* (2) observed that the maximum viscosity of evaporated milk is obtained when "the cooking time approaches the heat stability time." These observations of Deysher (2) and similar ones by Nelson (15) are supported by experience in commercial manufacture (7) that the viscosity obtainable during sterilizing is a function of the heat stability of the milk. The heat stability of milk varies greatly (1) and is determined mainly by salt balance (19). When stability of milk to heat is high, the evaporated milk is of relatively low viscosity, unless either temperature or time of sterilization or both are extended. Materially higher viscosity of milk sterilized by the conventional method (242° F. for 15 min.) as compared to that of the same milk sterilized by the HTST process emphasizes the fact that sufficient increase in viscosity takes place during sterilization only when denaturation of proteins by heat proceeds far enough to lower their stability and cause initial agglomeration of protein micelles. This treatment to obtain a greater viscosity results in poorer quality of milk from the standpoint of flavor and color.

This study presents the findings on the new method of obtaining optimum viscosity in evaporated milk, without any additional deleterious effect on color or flavor, by "conditioning" the proteins through enzymatic treatment prior to sterilization.

EXPERIMENTAL

Evaporated milk used in the experiments was secured from a commercial condensery. The milk was preheated, condensed, homogenized and canned under the usual standard commercial conditions at the condensery. For enzymatic treatment the samples were taken off the processing line just prior to sterilization and immediately placed in ice water and held at this temperature until the

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enzymatic treatment could be applied. In all experiments the milk was subjected to enzymatic action immediately or within 12 hr.

An aqueous solution of enzyme[2] of desired concentration was prepared just before use and added to the milk through a hole punched in the lid of the can. The addition of enzyme was facilitated by application of a suction tube leading from an aspirator to the second hole in the can. The effect of dilution was minimized by adding not more than 1 ml. of enzyme solution to any one 14.5-oz. can of milk. The cans were inverted ten times following the enzyme addition, sealed and incubated at desired and controlled time-temperature conditions. The temperature of milk at the time of addition of the enzyme was the same as the chosen temperature of incubation.

Immediately following the incubation period, the canned milk was sterilized in a Fort Wayne pilot batch sterilizer. The heating temperature-time, unless otherwise indicated, was as follows: 6 min. to 224° F., 5 to 6 min. from 224 to 242° F. controlled to give a rise of 3° F. per minute, 14 min. at 243° F. and 10 min. for cooling. Agitation was continuous during heating and cooling. This schedule of time-temperature heating was adopted because control (untreated) samples so sterilized in a pilot batch sterilizer were comparable, with respect to viscosity and degree of browning, to milk of the same batch sterilized in the commercial plant in a continuous sterilizer.

Samples designated as control in this study, as compared to treated samples, were cans of identical milk which underwent exactly the same treatment with respect to cooling or warming, incubation and sterilization, except that no enzyme was added.

Viscosity was measured at 25° C. by the Fisher Electroviscometer. The pH measurements were made with the Beckman glass electrode pH meter. The protein and non-protein nitrogen distribution was determined by the method as described by Rowland (16) and adapted to evaporated milk. Analyses for tyrosine value, color and fluorescence were made according to methods previously described (6, 21, 22).

RESULTS

The typical data showing the effect upon the viscosity of milk after sterilization by "conditioning" milk proteins prior to sterilization by added enzymes are shown in figures 1, 2, 3, 4 and 5.

The data in figure 1 illustrate the relationship between the increase in viscosity and the concentration of pepsin when milk was incubated at low temperature for various periods of time. The data in figure 2 indicate the effect of temperature of incubation on the rate of increase in viscosity at the same levels of enzyme. It is evident that in the initial stages of enzyme action on proteins of milk, at concentrations of enzymes as low as practiced in these experiments (in the case of pepsin, less than 1 ppm.), the increase in viscosity is directly related to the enzyme concentration, time and temperature of incubation, *i.e.*, the extent of initial proteolysis. The maximum viscosity that can be obtained in evaporated

[2] Powdered pepsin and pancreatin were of USP grade obtained from Pfanstiehl Chemical Co. Other enzymes were powderd preparations obtained from General Biochemicals, Inc.

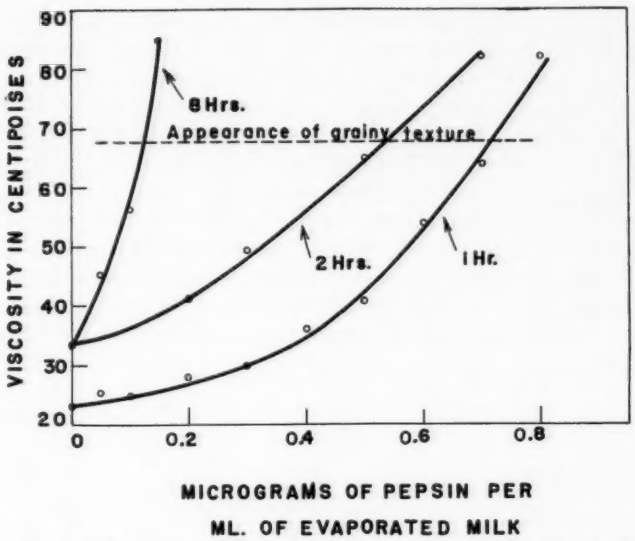

FIG. 1. Viscosity of evaporated milk as affected by the concentration of pepsin and the time of incubation at 38 to 40° F. Milk sterilized at 243° F. for 14 min.

milk by enzymatic treatment without causing the grainy texture is between 65 and 75 centipoises at 25° C. (77° F.), which is about twice as high as the average viscosity of experimental controls or commercial samples of evaporated milk

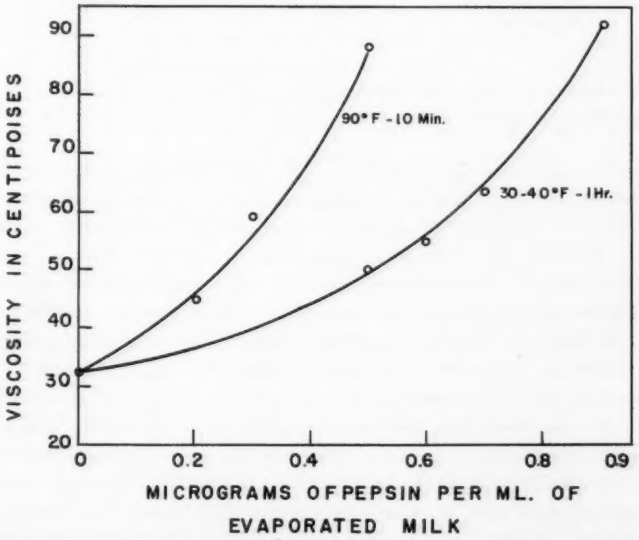

FIG. 2. Viscosity of evaporated milk as affected by temperature of enzymatic treatment. Milk sterilized at 243° F. for 14 min.

processed from the milk of relatively high stability to heat. More extensive proteolysis as effected by enzyme concentration, time or temperature of incubation lowers the heat stability of milk to the extent that it causes grainy texture and then coagulation. This is shown by the horizontal broken line in figure 1, indicating that evaporated milk of viscosity above the line was grainy as determined by the loop test, *i.e.*, examination of the film of milk under light. Enzymatically treated evaporated milk also was examined for feathering in coffee. The samples that had negative loop test gave a negative feathering test also.

Results similar to those obtained with pepsin and pancreatin as shown in figures 1, 2 and 4, respectively, were obtained employing other single or mixed proteolytic enzymes. The potency of these enzymes with respect to the increase in viscosity of evaporated milk was in decreasing order as follows: pepsin, pro-

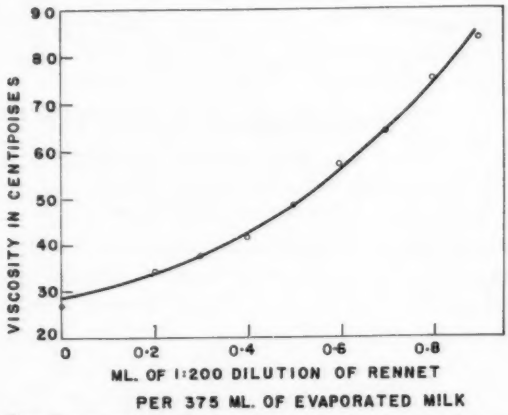

FIG. 3. Viscosity of evaporated milk as affected by treatment with rennet at 90° F. for 10 min. Milk sterilized at 243° F. for 14 min.

tease, trypsin, pancreatin and enzylac. The approximate concentrations of enzymes which were found to give an optimum viscosity at an incubation temperature of 38 to 40° F. for 1 hr. are as follows: pepsin—0.7 to 0.9 ppm.; protease—10 to 15 ppm.; trypsin—15 to 20 ppm.; pancreatin—50 to 75 ppm. The effect of addition of rennet is illustrated in figure 3. A commercial solution of rennet which is known to contain pepsin was used. With enzylac it was possible to obtain higher viscosity than 70 centipoises without formation of grain in the body of evaporated milk. However, enzylac preparations contain lipase in sufficient quantity to produce a distinctly rancid flavor in milk.

The enzyme concentration necessary to produce an optimum viscosity varies directly with the stability of milk to heat, and therefore varies from one batch of milk to another. Milk that already is on the borderline with respect to its stability to heat will have, as a rule, relatively high viscosity upon sterilization without further destabilization by enzymatic treatment. On the other hand, milk with high stability to heat or milk that has been overstabilized can be destabilized

by enzymatic treatment, thus making it possible to obtain an optimum viscosity upon sterilization without additional cooking, avoiding thereby an injury to the flavor and color of milk. The relation of enzymatic treatment to the stability of milk to heat is illustrated in figure 4. Increasing the stability of milk by addition of Na_2HPO_4 required the addition of a greater amount of enzyme—in this case pancreatin—to obtain the same increase in viscosity as in the milk without Na_2HPO_4 added. The stabilizer was added to milk at the rate of 13 oz. of $Na_2HPO_4 \cdot 12H_2O$ per 1000 lb of milk (fig. 4). Similar results were obtained with other enzymes and Na_2HPO_4. As would be expected, the action of Na_2HPO_4 is not an enzyme-inhibitory action, but rather one of raising the stability of milk to heat and thus reducing the viscosity

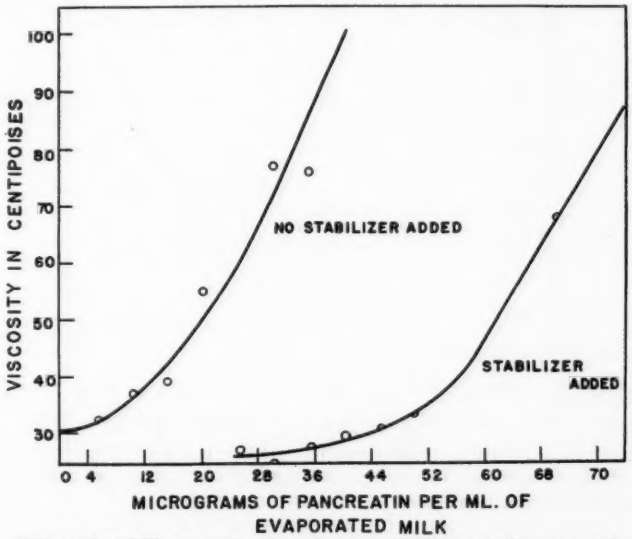

FIG. 4. Effect of stabilizer on viscosity of enzymatically treated evaporated milk. Incubation time, 30 min. at 98.6° F. Milk sterilized at 243° F. for 14 min.

under the equivalent heat treatment of sterilization. To counteract the action of Na_2HPO_4, it was necessary to carry on proteolysis to a greater extent by increasing the concentration of the proteolytic enzyme. Similarly, destabilization of milk to heat by incipient proteolysis, to the point where milk is coagulated upon sterilization, can be corrected by the addition of Na_2HPO_4 or another suitable stabilizer to milk after it has been subjected to proteolysis.

The critical feature and disadvantage of HTST sterilization of evaporated milk is low viscosity. The data in figure 5 show that a control sample of milk sterilized at 260° F. for 2 min.[3] had a viscosity of only 12.5 centipoises. The

[3] Samples were sterilized in a Fort Wayne pilot batch sterilizer by fixing the safety valve of the sterilizer to release steam at 30 lb. pressure The come-up time was 5–6 min. to 190° F., and 4 min. from 190 to 260° F.

data show further that samples of the same milk sterilized in the same time as the control, but treated with pepsin at 33 to 34° F. for 1 hr., showed progressive increase in viscosity. The sample having a viscosity of 80 centipoises was grainy, but all the rest of the samples were satsifactory. It is evident that by enzymatic treatment it is possible to increase substantially the viscosity of evaporated milk sterilized by the HTST method. The data on the increase in viscosity in this case are similar to the data obtained by conventional sterilization (243° F. for 14 min.), except for the greater amount of enzyme that needs to be added to obtain a comparable viscosity.

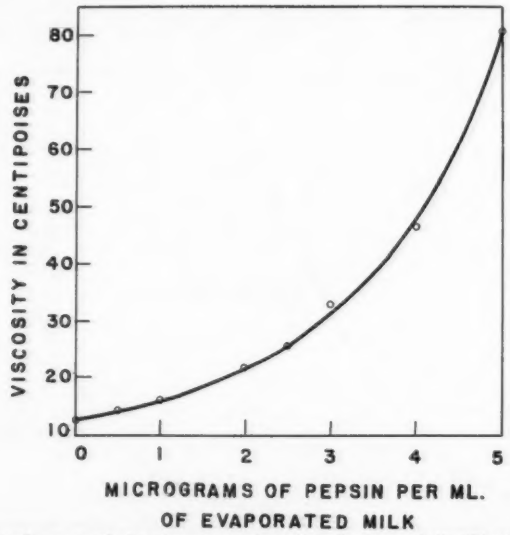

FIG. 5. Effect of enzymatic treatment on viscosity of evaporated milk sterilized at 260° F. for 2 min. Incubation time, 1 hr. at 34° to 36° F.

The results illustrated in figures 1, 2, 3 and 4, which were obtained by sterilization on a Fort Wayne batch sterilizer, were fully confirmed by several experiments on enzymatic treatment in commercial plants, with sterilization of samples in a continuous sterilizer. In one plant the method of addition of enzyme was varied by adding the water suspension of enzyme to cans in the line before filling, and using the interval of time that elapses between filling and sterilization as the incubation time. The results were equally satisfactory and similar to those obtained by the addition of the enzyme to milk through holes punched in cans, as has been described in the experimental procedure.

With respect to the other characteristics such as color, fluorescence, flavor, pH, titratable acidity, soluble nitrogen and formol titration, the enzymatically treated evaporated milk at the level of treatment necessary to obtain a maximum viscosity without coagulation showed no significant difference as compared to control, *i.e.*, the same milk not enzymatically treated but processed otherwise in the same way

and at the same time. Average flavor scores and criticisms by experienced judges indicated that enzymatically treated samples had slightly less caramelized flavor than the control. This difference may be due to a higher viscosity of milk *per se*, or due to a decreased rate of browning reaction because of higher viscosity attained by the enzymatically treated milk during sterilization.

The data show that slight proteolytic changes do markedly affect the heat stability of milk. Addition to stable milk of 1 ppm. or more of pepsin and incubation at such a low temperature as 38° F. for 1 hr. generally is sufficient to cause coagulation of milk during sterilization. Additional data on the lowering of stability of milk to heat by initial proteolysis are presented in table 1. Coagulation time was determined by heating milk in specially constructed glass pressure flasks in a thermostatically controlled oil bath at 240° F. Prior to heating, a suspension of enzyme was added and the milk was incubated at 38° F. for 1 hr.

TABLE 1

The effect of incipient proteolysis in unsterilized evaporated milk on its stability to heat

Enzyme	Concentration of added enzyme	Time of coagulation	Enzyme	Concentration of added enzyme	Time of coagulation
	(γ/ *ml. of milk*)	(*min.*)		(γ/ *ml. of milk*)	(*min.*)
None	Control	30	None	Control	26
Pepsin	0.8	18	Pancreatin	60	16
Pepsin	1.0	14	Pancreatin	75	12
Pepsin	5.0	3.0	Pancreatin	150	2.0

Stability of milk to heat is lowered by initial enzymatic hydrolysis of milk proteins. Lowering of heat stability of milk by bacterial enzymes independent of acid production was observed by Frazier in 1925 (4), who attributed the effect only to "rennin-like enzymes" produced by bacteria.

DISCUSSION

Subjecting evaporated milk to a very slight and limited enzymatic proteolysis produces a marked increase in the viscosity of milk upon sterilization. The increase in viscosity is directly related to the lowering of stability of milk to heat by the enzymatic action on the proteins of milk in the initial stages of proteolysis. The extent of enzymatic action (as determined by enzyme concentration, time and temperature of incubation), which is necessary in order to obtain a maximum increase in viscosity for any given batch of milk, therefore depends upon the initial stability of milk to heat, as well as upon the time and temperature of sterilization. In all cases, however, only minute quantities of enzyme need be added to obtain the maximum increase in viscosity. The enzyme preparations used in this work were of only relative purity; with crystalline enzyme preparations the required quantities are much smaller. As this is being written, the experiments with crystalline pepsin[4] show that only 0.003 ppm. of the enzyme

[4] Pepsin (2X crystallized) obtained from General Biochemicals, Inc., O.

was necessary to add with incubation at 32 to 34° F. for 1 hr. to effect a viscosity of 64 centipoises, as compared to 29.6 centipoises for a control. Considering the minute concentration of enzyme and the short period of incubation at a temperature and pH far from optimum enzyme action, the question arises as to the nature of changes in the protein molecule initiated by enzymatic action, which affect so markedly the physical characteristics of milk upon sterilization by heat.

At the level of enzyme concentration used, there was no significant difference in soluble nitrogen or formol titration in treated samples as compared to the control. Tyrosine value, which has been shown to be a very sensitive method (6, 21) for detection of minute amounts of protein hydrolysis, was slightly but consistently higher for treated samples as compared to the control. The lack of gross chemical changes in the protein molecule, upon enzymatic treatment even somewhat more extensive than that used in this study, was observed also by Keil and Roundy (9), Hull (6) and Storrs (20). Storrs (20), in the study of milk modification with pancreatic enzymes for lowering the curd tension of milk, advanced the theory that the proteolytic effect is only upon the surface of the protein, leaving the protein molecule essentially unaltered except for modifying the surface properties with respect to stability and coagulating characteristics of the protein. Keil and Roundy (9) found no difference in osmotic rates in enzylac-treated low curd-tension milk, as compared to the same but untreated milk, and they attributed the enzymatic effect to "conditioning" of the molecules of casein by a weakening of their internal structure, rendering them susceptible to dissociation by heat. Keil and Roundy's (9) postulation that "physical measurements rather than chemical methods seem to have greater possibilities for measuring this effect" is well borne out by the present finding of marked increases in viscosity upon heating of enzymatically treated milk.

It is evident that in "conditioning" of milk proteins by proteolytic enzymes the resulting effect, which Keil and Roundy (9) call a "weakening of the internal molecular structure," must involve other bonds than the hydrolysis of peptide bonds. It is well established that casein is a globular type of protein (3, 8, 13). The structure of globular protein is considered as consisting of peptide chains, possibly linked through disulfide bonds and held in an orderly coiled configuration by ionic and hydrogen bonds. The breaking of these bonds, as in the process of denaturation, leads to the uncoiling of molecules and to an increase in the chemical reactivity due to exposure of reactive groups previously hidden away from the surface and not reacted upon. The unfolding of the globular protein will result in greater asymmetry of the particles as well as in increase of hydration due to the increase in number of polar groups, thus increasing considerably the viscosity of the protein solution. Furthermore, the presence of bivalent cations such as Ca^{++} in milk will permit the formation of intermolecular salt bridges between free (exposed at the surface) carboxyl and amino groups, leading to the formation of larger aggregates followed by coagulation of the protein.

While there is no doubt that an extensive proteolysis consists mainly of the cleavage of peptide bonds, the nature of the changes in protein molecule brought about by an initial enzyme action, such as has been used in this study in "condi-

tioning'' of milk proteins, is not clear at present. On the basis of experimental evidence involving measurements of volume changes (12), viscosity (8), ultracentrifugal and electrophoretic properties (14), the Linderström-Lang theory of denaturation of proteins as the initial effect of enzyme action (10, 11) finds the greatest support. In the case of some globular proteins the action of an enzyme as a denaturase in the incipient stages of proteolysis appears to be established as resulting in unfolding of the protein (5, 8, 14). This means that the effect of initial reaction in proteolysis is similar or identical to the effect of denaturation by heat, where the action is on hydrogen and ionic bonds permitting the uncoiling and displacement of the peptide chains to the surface. In such a case, whether the denaturation of globular proteins is accomplished by heat or by an incipient proteolysis, there is an increase in hydration of proteins and the increase in viscosity of protein solution. Jirgensons (8) has shown that initial degradation of casein by enzymatic action is accompanied by an increase in viscosity. Further degradation involving hydrolysis of —CO—NH— bonds is followed by a sharp decline in viscosity.

The experimental results of this study showing the lowering of stability of milk to heat, increase in viscosity and finally stabilization by the addition of Na_2HPO_4 to milk, which has been destabilized by enzymatic treatment, are fully explained in the opinion of the authors by the theory, and perhaps best by this theory, that the effect of incipient proteolysis in milk is protein denaturation similar to, or identical, in major respects, with denaturation by heat. If this theory is correct then other changes in milk that are brought about by action of heat besides those already mentioned also should be obtained by an action of initial proteolysis. Two well known phenomena, (a) lowering curd tension of milk and (b) inhibition of the development of oxidized flavor, are accomplished equally well either by heat or by an initial proteolysis. An increase in water sorption by milk proteins upon the heating of milk in its application to improvement in bread should also be obtained by enzymatic treatment of milk as well as by heat. Preliminary and unpublished data (17) indicate this supposition also is true. There are further ramifications of this theory, with respect to continuous changes of the properties of milk on aging. Such changes are due to incipient proteolysis and therefore gradual denaturation of proteins; this effect is brought about by proteolytic enzymes of bacteria as a normal function of bacterial metabolism in milk.

CONCLUSIONS

Proteolytic enzymatic treatment of evaporated milk within the limits of what generally is regarded as the first phase of enzyme action increases the viscosity of evaporated milk upon its sterilization.

Enzymatic treatment lowers the stability of milk to heat. The state of milk proteins as affected by incipient proteolysis is a factor in the stability of milk to heat in addition to the factor of salt balance.

The viscosity of evaporated milk with relatively high stability to heat can be increased to maximum (more than doubled) by enzymatic treatment without

deleterious effect on color or flavor of milk, as contrasted to the common method of increasing viscosity by additional cooking in the process of sterilization.

The method of increasing viscosity of evaporated milk by enzymatic treatment prior to sterilization is applicable to both the standard and short-time high-temperature sterilization processes, but enzymatic activity in the case of HTST process has to be greater to obtain the same increase in viscosity.

Milk destabilized to heat by enzymatic treatment can be made stable to heat again by the addition of such stabilizing salts as Na_2HPO_4.

The effects of incipient proteolysis with respect to viscosity and heat stability of milk are explained in the light of the Linderström-Lang theory of denaturation as the mechanism of initial enzyme action in globular protein systems.

The results of the present study and other phenomena effected by an incipient proteolysis of milk proteins, such as the inhibition of development of oxidized flavor, lowering of curd tension and increased water sorption, can be adequately explained on the assumption that denaturation is an initial reaction in proteolysis. Since all above phenomena can be produced as well by the heating of milk, it is advanced that enzymatic denaturation of the proteins in milk is similar in its major respects to heat denaturation, *i.e.*, the breaking of ionic and hydrogen bonds through which the peptides are linked, thus permitting the coiled peptide chains to uncoil.

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THE VALUE OF DISTILLERS FEEDS FOR MILK PRODUCTION

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Distillers by-products are popular as a feed ingredient in dairy rations, and they have been widely used for years. Distillers dried grains were marketed as dairy feed in New England before 1900. It is somewhat surprising, therefore, that so few studies have been made to compare the value of distillers feeds with other common feedstuffs for milk production. While few comparisons have been reported involving corn distillers dried grains, only one preliminary test appears to have been made with rye distillers dried grains for dairy cows. Preliminary studies have shown that distillers solubles as a part of concentrate mixtures are palatable for dairy cows, but little is known about their value in replacing other feeds. The present estimated values of these feeds are based largely upon their chemical composition and feeding value for beef cattle, rather than upon their tested usefulness for milk production.

Hills (4) conducted a series of tests in which corn distillers dried grains were compared with a mixture of corn meal and wheat bran. Cows fed the corn distillers dried grains produced about 15 per cent more milk and 18 per cent more fat than the control animals. A consistent increase in the fat content of the milk resulted from feeding corn distillers dried grains when the test periods were 4 wk. or less in length but not when the periods were 5 wk. or longer, and he concluded, therefore, that these feeds produced only a temporary increase in the fat content of milk. Lindsey (5) compared corn distillers dried grains with corn gluten feed using two groups of three cows each. The concentrate portion of the ration was made up of 3.0 lb. wheat bran and 3.7 lb. of either corn distillers dried grains or corn gluten feed. As an average during the last 4 wk. of the 6-wk. test periods, the cows fed the distillers grains produced 1.5 lb. more milk daily and consumed 6.1 lb. less dry matter to produce 100 lb. of milk than those fed corn gluten feed. Hills (4) observed that on equal feed intakes cows produced about 4 per cent more milk and 6 per cent more fat on corn distillers dried grains than on brewers grains. The brewers grains were approximately equal to a mixture of linseed meal and cottonseed meal. Armsby and Risser (1) reported that feeding 3.5 lb. of corn meal plus 5.5 lb. of corn distillers dried grains as a supplement to hay and corn silage resulted in significantly more milk and fat than 6.0 lb. corn meal plus 3.0 lb. cottonseed meal. They attributed the extra milk and fat yields from the distillers grains to the higher fat content. Hart and Humphrey (3) showed that the protein furnished by distillers dried grains was equal in value for maintaining nitrogen balance and milk production in lactating dairy cows to linseed meal, casein, dried skimmilk, cottonseed meal and gluten feed when clover or alfalfa hay was the roughage. When corn stover was the only roughage, distillers dried grains proved superior to corn gluten feed as the protein supplement.

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Hills (4) published preliminary data from two cows which showed rye distillers dried grains were worth 6 per cent less for milk production and 9 per cent less for butterfat yield than corn distillers dried grains. No other comparisons of these feeds appear to have been made with milking cows.

Sutton (8) studied the value of corn distillers dried solubles using ten pairs of milking cows in a 12-wk. feeding experiment. He reported that 100 lb. of corn distillers dried solubles were approximately equal to 73 lb. of soybean oil meal, containing 41 per cent protein, 13 lb. of corn meal and 13 lb. of ground oats.

Studies of the value of distillers feeds now in use appear to be needed, especially because manufacturing differences may have caused nutritional variations from products studied years ago.

EXPERIMENTAL

Two experiments have been carried out to determine the value of distillers feeds for milk production. In both tests, corn distillers dried solubles and corn distillers dried grains with solubles were compared with corn gluten feed as protein supplements in concentrate mixtures for dairy cows. In the second test, rye distillers dried grains with solubles also were studied. The concentrate mixtures used in the studies are shown in table 1.

TABLE 1

Composition of the concentrate mixtures

Ingredients	Mixture no.			
	I	II	III	IV
Ground barley	500	500	500	500
Ground oats	100	100	100	100
Corn gluten feed	310	.....	.....	.....
Corn distillers dried grains with solubles	.....	385	.....	.....
Corn distillers dried solubles	.....	.....	335	.....
Rye distillers dried grains with solubles	.....	.....	.....	235
Soybean oil meal, solvent extracted	75	.....	50	150
Dicalcium phosphate	10	10	10	10
Salt	5	5	5	5
Total	1,000	1,000	1,000	1,000

In mixture II, 38.5 per cent of corn distillers dried grain with solubles served as the only protein supplement to barley and oats. In the other concentrates, soybean oil meal and corn gluten feed or the distillers feeds were combined to furnish an amount of protein equal to that from the corn distillers dried grains with solubles in mixture II. The corn distillers dried grains with solubles and corn distillers dried solubles were from a mash composed of 78 per cent corn, 12 per cent rye and 10 per cent barley malt. The rye distillers dried grains with solubles were from a mash composed of 90 per cent rye and 10 per cent barley malt.

The chemical composition of the concentrate mixtures and of the silages and hays fed is shown in table 2. The silage was only fair in content of ears. The hays used in both studies were cut July 14 to 20, and were rather poor in quality.

Holstein cows which had recently passed their maximum milk production but had not been rebred were selected for the study. They were fed the same roughages that were to be used in the experiment and a standard concentrate mixture during a preliminary period of a month or more to observe levels of production and to adjust feed intakes. Then they were grouped into replicates of similar production and the cows in each replicate allotted at random to the treatments to be compared in the first period. The Latin square was used as the experimental design in both tests. The experimental periods were 6 wk. long in the first study and 4 wk. in the second, and each cow was fed each mixture in the course of the study. The cows were fed an amount of corn silage which they would consistently consume. Hay was allowed in excess of the intakes to permit daily weighbacks of 3 to 4 lb. Concentrates were fed at a rate of approximately 1 lb. for

TABLE 2

Chemical composition of feeds

Feedstuff	Moisture	Protein	Fat	Crude fiber	N.F.E.	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
		Experiment I				
Concentrate mixtures:						
I. Corn gluten feed	12.7	18.1	2.1	5.7	54.5	6.9
II. Corn distillers grains	12.5	16.7	4.2	6.1	54.5	6.0
III. Corn distillers solubles	13.6	17.3	4.3	4.6	52.1	8.1
Corn silage, fair in ears	73.8	3.6	0.9	8.9	11.3	1.5
Mixed timothy and clover hay	8.6	9.4	1.8	34.0	41.2	5.0
		Experiment II				
Concentrate mixtures:						
I. Corn gluten feed	12.0	18.9	2.3	5.8	54.2	6.8
II. Corn distillers grains	12.2	18.8	4.8	6.0	52.0	6.2
III. Corn distillers solubles	11.8	18.8	4.1	4.5	52.8	8.0
IV. Rye distillers grains	11.9	18.8	3.1	7.2	51.4	7.6
Corn silage, fair in ears	72.0	3.8	1.0	8.8	13.0	1.4
Timothy hay	9.0	7.5	2.0	35.0	42.5	4.0

each 3.5 lb. of 4 per cent fat-corrected milk (F.C.M.) produced during the previous week. The cows were changed abruptly from one concentrate mixture to another and no refusal of the concentrate mixtures occurred.

RESULTS AND DISCUSSION

In calculating the milk production, the first week following a change in ration was considered as transition and was not included in the averages. The average milk and fat yields are summarized in table 3.

In the first study the cows produced daily 3.1 lb. more 4 per cent F.C.M. on the mixture containing corn distillers dried grains with solubles and 2.5 lb. more on the corn distillers dried solubles than on the mixture containing corn gluten feed. The results of experiment II agreed fairly closely with experiment I. The cows produced 2.1 lb. more 4 per cent F.C.M. on the concentrate mixture containing corn distillers dried grains with solubles and 0.8 lb. more on the one with corn distillers solubles than on the mixture containing corn gluten feed.

TABLE 3

The average daily milk and fat production

Mixture containing	Milk	Test	Fat	F.C.M.[a]
	(*lb.*)	(%)	(*lb.*)	(*lb.*)
Experiment I (*12 cows*)				
Corn gluten feed	41.9	3.78	1.54	39.3
Corn distillers dried grains with solubles	44.1	3.76	1.65	42.4
Corn distillers dried solubles	43.1	3.84	1.64	41.8
Experiment II (*8 cows*)				
Corn gluten feed	30.8	3.45	1.07	28.3
Corn distillers dried grains with solubles	32.8	3.49	1.15	30.4
Corn distillers dried solubles	32.0	3.40	1.08	29.1
Rye distillers dried grains with solubles	31.0	3.46	1.08	28.5

[a] 4% F.C.M. (Gaines, 2).

The average daily production was practically the same on rye distillers dried grains with solubles as on corn gluten feed.

Analysis of variance failed to show that these differences in the production of 4 per cent F.C.M. were statistically significant in either experiment. When the results were combined, the differences approached significance at the 5 per cent level. Considering the consistent results and the size of the differences, it seems logical to place some reliance on the estimate that corn gluten feed is probably not as efficient as a protein supplement to barley and oats for milk production as corn distillers dried grains.

The corn distillers grains studied were considerably higher in fat content than the corn gluten feed. The rye distillers grains were intermediate in fat content. It is possible that the higher fat content of the distillers feeds explains the higher production on these feeds in line with earlier studies (Maynard and McCay, 7; Loosli *et al.*, 6), but other factors also may be involved.

The average daily feed intakes and gains in weight and the average body weights of the cows on the various concentrate mixtures are summarized in table 4. Shown in table 5 are the amounts of feeds consumed for each 100 lb. of

TABLE 4

The average daily feed intakes and changes in body weight

Mixture containing	Concentrate	Silage	Hay	Body wt.	Wt. gain
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Experiment I					
Corn gluten feed	15.3	47	9.4	1,344	0.2
Corn distillers dried grains with solubles	16.4	47	9.1	1,352	0.8
Corn distillers dried solubles	15.4	46	9.4	1,361	0.6
Experiment II					
Corn gluten feed	10.2	36	10.2	1,119	0.2
Corn distillers dried grains with solubles	10.2	37	10.3	1,125	0.3
Corn distillers dried solubles	10.4	36	10.6	1,106	0.5
Rye distillers dried grains with solubles	10.2	38	10.7	1,116	0.9

4 per cent F.C.M. produced on the different concentrate mixtures. The cows required more concentrates, silage and hay to produce each 100 lb. of milk on the mixture containing corn gluten feed than on those containing corn distillers feeds, and they gained less in body weight. These comparisons show that the corn distillers feeds studied were somewhat more valuable as protein supplements in concentrate mixtures for milking cows than a mixture of corn gluten feed and solvent-extracted soybean meal.

SUMMARY

In two experiments Holstein cows produced an average of 2.6 lb. more 4 per cent fat-corrected milk daily on a concentrate mixture containing corn distillers dried grains with solubles and 1.6 lb. more with corn distillers dried solubles than on a similar mixture containing corn gluten feed and soybean oil meal as the principal protein supplement to oats and barley. Rye distillers dried grains with solubles gave results approximately equal to corn gluten feed in a single study.

TABLE 5

The feed intakes and gain in body weight per 100 lb. of 4% milk

Mixture containing	Feed/100 lb. of 4% milk			Gain in wt.
	Concentrate	Silage	Hay	
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Experiment I				
Corn gluten feed	38.9	120	23.9	0.5
Corn distillers grains with solubles	38.7	111	21.5	1.9
Corn distillers solubles	36.8	110	22.5	1.4
Experiment II				
Corn gluten feed	36.0	127	36.0	0.7
Corn distillers grains with solubles	33.6	122	33.9	1.0
Corn distillers solubles	35.7	124	36.4	1.7
Rye distillers grains with solubles	35.8	133	37.5	3.2

Analyses of variance of the results of the two studies showed that the differences in production approached significance at the 5 per cent level. The authors believe the results suggest that corn gluten feed probably is not as efficient as a protein supplement to barley and oats for milk production as corn distillers dried grains.

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STUDIES ON THE USE OF PERMANENT MILK PIPELINES IN DAIRY BARNS. I. EFFECT OF DIFFERENT TYPES OF INSTALLATION ON MILK QUALITY

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Within the past 25 yr. the practice of milking cows by machine has become almost universal. To a large extent milk-handling procedures have been standardized to include (a) the manual removal of milk from the stable, (b) cooling by use of surface coolers and (c) storage in cans held under various conditions of refrigeration. The efficiency of such methods has practically reached its peak within the limits of the systems so far developed. Therefore, if labor efficiency is to be improved and product quality maintained, new methods must be developed for milking and milk handling at the farm level.

Recent developments in milk-handling operations have included the use of cold-wall collection and storage tanks, as well as the use of milk pipelines. In this connection, Amerding (1) reported that tanks were more satisfactory than cans for storing milk because they (a) gave greater cooling efficiency and thereby lowered rejections for high-temperature spoilage, (b) lessened air-borne contamination, (c) eliminated contact with undesirable metals, (d) reduced spillage and stickage losses and (e) afforded better control by the dairy inspector. Bartlett (3) observed that milk quality was improved and transportation and receiving cost materially reduced by farm use of cold-wall tanks. Similarly, Clement (5) reported that shipping costs were lowered more than 50 per cent when milk was shipped in tanks rather than in cans. Others (4, 7) have indicated the need for further investigation of the economic problems of milk production and marketing of the raw product.

Milk pipelines have been in limited use in dairy barns for a number of years. However, in practice they usually are dismantled for cleaning after each use. Thus, the possible advantages of labor reduction by carry away in pipelines is reduced. If permanent dairy barn pipelines can be cleaned in place as effectively as those in dairy plants (6), their use may result in considerable labor economy in milking operations.

The effect of various systems of milking and milk handling on quality and economy of production has been studied at this Station. This report presents the results of experiments designed to measure the influence of four systems of milking and milk handling on product quality. The data pertaining to the economy of production will be presented in a future communication.

EXPERIMENTAL

In this investigation four systems of milking and milk handling were studied, using two intra-group systems for comparison. These systems were studied concurrently from March 20, 1950, to September 25, 1950.

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In group 1 an open-combine system was compared with a completely closed-combine system. Both systems were installed in a three-stall in-line parlor with the stall floor 30 in. above the operator's pit. In the open-combine system the milk passed from the cow through the milker claw assembly and rubber milk tube into a Pyrex glass weigh jar suspended from a scale for automatic weighing. As soon as the milk weight was recorded and a sample taken for the fat test, the milk was released into a stainless steel pipe and drawn by vacuum through a Pyrex glass releaser jar, from which it was carried by gravity through another stainless steel pipe and delivered into the adjacent milk-handling room. There was about 1 ft. of clearance at the end of this pipe where the milk was exposed to the air of the milk room while dropping into an open strainer placed in a 10-gal. milk can. The herd milked by this system included 21 cows, which yielded an average of five to seven cans of milk per milking. Each can was set aside as it was filled and, at the conclusion of the milking operation, was submerged in ice-water contained in a wet-tank cooler. In this manner the milk was cooled to 46° F. in about 1.5 hr.

The completely closed-combine system, which was used in group 1 differed from the open-combine system in that: (a) it did not utilize the releaser jar to break its vacuum; (b) while it used stainless steel pipe to convey the milk from the milking parlor, this pipe was connected with hard rubber joints instead of stainless steel connections; (c) the milk, after being delivered to the milk room, remained under vacuum and was delivered directly into the cans which were connected in a series, submerged to the necks before the milking operation was started. The same cooler referred to above was used. The milk temperature was lowered to 46° F. in about 30 min. after the conclusion of the milking operation; and (d) in the closed-combine system the milk was strained by an in-the-line strainer installed just in front of each weigh can. These weigh cans were of stainless steel.

The method of cleanup was identical for both systems. At the conclusion of the milking operation, lukewarm water was flushed through the teat cup assembly, weigh cans and milk lines. Then the entire system of milker units and pipelines was dismantled and washed in a hot detergent solution with all steel and rubber parts brushed thoroughly inside and out. After they were washed, all parts were rinsed with clear water and then with a chlorine solution at a strength of 200 ppm. The weigh cans, milker heads, pipes and connections were stored on a drying rack until the next milking. The teat cups were reassembled and stored on a solution rack and filled with an 0.5 per cent lye solution. After the combine was reassembled for the next milking, it was rinsed in place with a chlorine solution of 200 ppm.

Immediately before milking, the udder was washed with a chlorine solution (200 ppm.) and, between cows, the teat cup assembly was dipped in a chlorine solution of the same strength.

In group 2 a conventional milking machine consisting of four units was compared with a cow-to-cooler combine system designed to carry the milk from the cow in her regular stall directly to the cooler without exposure to air. Twenty-four cows were used in this study. The two systems were alternated bi-weekly from

March 11, 1950, to May 29, 1950, when they were alternated weekly until September 25, 1950, the conclusion of the investigation.

The conventional system used four milker units with two men operating the milkers and one man carrying the milk. The milk from each cow was immediately poured from the milker unit into a partially covered pail and carried to the milk-handling room where it was weighed, sampled and strained into a stainless steel receiving vat. From this vat the milk was pumped over a tubular surface cooler and chilled to 50° F. before being delivered by gravity into an open milk can. Immediately after filling, each can was placed in a walk-in cooler for storage at 40° F. until delivery to the plant. The tubular surface cooler was covered with a stainless steel shield.

The milking machines were cleaned in the following manner: Immediately after milking they were flushed with cold water, after which they were dismantled completely and washed thoroughly in a hot detergent solution. All the equipment except the cooling tower and receiving vat was stored in a continuous dry heat cabinet at 160° F. The cooling tower and receiving vat were rinsed with cold water immediately after use, brushed with a hot detergent solution and sterilized with a chlorine solution of 200 ppm. Before the next milking the milker units, carry pails and cans were rinsed with a chlorine solution of 200 ppm. The cooling tower, receiving vat and pump assembly likewise were rinsed immediately before use.

The cow-to-cooler combine system was installed with permanent pipelines which were cleaned in position during the entire phase of this study, from March 11, 1950, until September 25, 1950. Two different lines were used—one of stainless steel in operation from March 1 until September 2, and one of Pyrex glass from September 5 until September 25. These lines, 200 ft. in length, were arranged in a complete circuit around the stanchion lines on the two sides of the barn and ended in a 5-gal. Pyrex glass surge chamber which broke the vacuum. Three-way valves were placed in the line so that the cleaning solutions could be propelled in either direction. The stainless steel and Pyrex glass were installed in such a way that they could be alternated and either put into use. The milk was drawn from the cow and drawn by vacuum through the pipeline to the surge chamber. From this point it was pumped a distance of 15 ft. through a stainless steel pipe into a 100-gal. stainless steel cold-wall tank located in the milk-handling room. The evening milk was held in this tank where it was cooled to 38° F. within 30 min. after it was drawn from the cows. A continuous record of the temperature was made with a Taylor recording thermometer. The morning milk was mixed directly with that produced the preceding evening, cooled and held until about 8 a.m., at which time it was canned off by vacuum for delivery to the plant in conventional milk cans. The temperature of the combined milk did not rise above 50° F. The milker units were disassembled, washed with detergent solution and stored in a dry heat cabinet at 160° F. Before the next milking they were reassembled and rinsed with a chlorine solution. The pipeline was cleaned immediately after each milking as follows: (a) Flushed with clear water at about 90° F. until clear of milk; (b) flushed with 20 gal. of an alkaline wetting agent at 140° F. An acid detergent was used every fourth milking; (c) sterilized

with 12 gal. of water at 185° F. Before each milking, the lines were flushed with 20 gal. of chlorine solution (200 ppm.). The 15 ft. of line between the surge chamber and cold-wall tank was taken down and cleaned following each milking. The cold wall tank was cleaned and sterilized once a day and rinsed with a chlorine solution before the evening milking.

The evening milk was refrigerated overnight and sampled just prior to the addition of the morning milk. After combining, the milk was thoroughly mixed and another sample was taken. Samples were removed from storage containers by use of a sterilized saucer-type stirrer. The 150-ml. (approximate) samples were collected in sterilized 0.5-pt. bottles which then were packed in ice for transport

TABLE 1

Average standard plate counts for open and closed combine systems

Period[a]	Group 1 cows							
	Combine open				Combine closed			
	Evening		Morning		Evening		Morning	
	No. of observations	Count/ml.	No. of observations	Count/ml.	No. of observations	Count/ml.	No. of observations	Count/ml.
1	5	13,000	5	14,000	6	4,800	6	4,950
2	5	3,750	5	5,750	13	7,700	13	7,800
3	3	4,500	3	4,350	7	3,650	7	4,750
4	10	16,000	10	18,000	8	2,250	7	2,350
5	9	16,000	9	30,000	5	3,500	5	3,700
6	8	10,500	8	15,000	8	3,450	8	3,700
7	9	15,500	9	14,500	7	25,000	7	12,500
8	9	9,900	9	12,500	2	6,100	2	6,700
9					6	7,600	6	7,000
Av.		11,000		14,000		7,100		5,600

[a] The 9 periods of observations extended from March 11, 1950, to Sept. 25, 1950, and each period covered approximately 2 wk.

RESULTS

to the laboratory. The samples were examined within 4 hr. The sanitary quality of the milk was determined by the standard plate count procedure (2) and the results are reported as the logarithmic averages (9) in order that quality trends may be evaluated. An analysis of variance (8) was made to determine the statistical significance of difference between the four systems.

Table 1 shows the average plate count for the different periods involved. An analysis of variance shows a significant difference between the two systems in group 1. The plate count in the open-combine system was approximately double that found with the closed-combine system. However, it should be noted that each system produced milk well within the accepted bacteriological standard for Grade A. The higher count for the open-combine system may in part be accounted for by delayed cooling experienced under this system—a difference of 1 hr.

The results obtained from the two systems studied in the second group are shown in table 2. An analysis of variance showed no significant difference. The milk from both systems had low plate counts, the conventional system having

average counts of 4,850 for the evening sample and 5,100 for the morning composite sample of evening and morning drawn milk. The pipeline cow-to-cooler combine had average counts of 8,750 for the evening sample and 8,075 for the morning composite sample. The plate counts for the individual periods of observation were within the limits acceptable for Grade A milk. The averages of 39,000 and 17,000 during the fourth period for the pipeline cow-to-cooler combine were considerably higher than were the averages during the other periods. These comparatively high averages occurred during a breakdown in cleaning procedure. During this period, the daily counts ran over 300,000 for 4 days. It is significant to note that a return to the prescribed routine for cleaning the

TABLE 2

Average standard plate counts for pipeline and conventional milking systems

Period[a]	Group 2 cows							
	Conventional				Pipeline cleaned in position			
	Evening		Morning		Evening		Morning	
	No. of observations	Count/ml.	No. of observations	Count/ml.	No. of observations	Count/ml.	No. of observations	Count/ml.
1	5	1,300	5	1,550	11	4,350	11	6,900
2	5	1,400	5	2,050	8	1,600	8	2,000
3	4	2,050	4	5,200	3	1,600	3	2,100
4	8	6,700	8	11,500	6	39,000	6	17,000
5	4	4,100	4	3,700	10	3,600	10	3,800
6	7	8,000	8	7,550	9	7,100	9	11,000
7	10	9,200	10	4,550	9	4,500	9	5,700
8					9	5,100	9	8,200
9					14	12,000	14	16,000
Av.		4,850		5,100		8,750		8,075

[a] The 9 periods of observations extended from March 11, 1950, to Sept. 25, 1950, and each period covered approximately 2 wk.

pipeline was followed by a drop to the average count of 3,600 and 3,800, respectively, for the evening and combined samples. This drop in bacterial count was achieved without dismantling the line. A return to the regular cleaning procedure was sufficient.

Table 3 shows the range in actual plate counts. Using the conventional system, 96.51 per cent of the observations showed actual standard plate counts under 50,000. When using the permanent pipeline system, there were only 14 cases out of 159 observations where the count was above 50,000. The single count above 300,000 was for an evening sample. The composite sample taken the next morning showed a count of only 20,000, indicating a possible error of sampling.

In order to test the effect of different personnel as a factor in the bacterial count, averages were computed for six different barn attendants. These results are shown in table 4. They indicate that personnel may be a factor of considerable importance. In comparing attendants 1 and 2, it is observed that the average plate count achieved under attendant 2 was about twice as high as for attendant 1. Also, the counts achieved under attendants 3, 5 and 6 were ex-

tremely high when compared with attendants 1, 2 and 4. While these data are too limited to draw specific conclusions, they do indicate that personnel may be an important factor in determining quality of milk.

TABLE 3

Range in standard plate count by seasons

Range of count /ml.	Conventional milking						Milking through permanent pipelines					
	No. of observations						No. of observations					
	Spring	%	Summer	%	Fall	%	Spring	%	Summer	%	Fall	%
0– 5,000	26	81.25	18	34.62	0		49	65.33	14	25.45	3	10.34
5,001– 10,000	6	18.75	16	30.77	1		3	4.00	25	45.45	9	31.03
10,001– 15,000	0		10	19.23	0		4	5.34	1	1.83	5	17.24
15,001– 20,000	0		3	5.77	0		6	8.00	0	0.0	2	3.45
20,001– 25,000	0		0	0.0	0		2	2.67	1	1.82	2	3.45
25,001– 30,000	0		0	0.0	0		2	2.67	0		3	10.34
30,001– 35,000	0		0	0.0	0		2	2.67	0		1	6.91
35,001– 40,000	0		1	1.92	0		1	1.33	0		0	
40,001– 45,000	0		1	1.92	1		2	2.67	0		0	
45,001– 50,000	0		1	1.92	0		1	1.33	0		1	6.90
50,001–100,000	0		2	3.85	0		2	2.67	3	5.45	3	10.34
100,001–300,000	0		0		0		0	0.0	11	20.00	0	
300,001–400,000	0		0		0		1	1.32	0		0	
Totals	32		52		2		75		55		29	

Table 5 shows the effect of outside daily maximum temperature on the quality of milk produced. The results reported here indicate a slight tendency toward a rise in the average bacterial count with increasing outside maximum temperature for the conventional and the open-combine systems, but not for the other two. This difference probably was associated with the delayed cooling in these systems. With the conventional system, cooling was delayed until several cows were milked and a second operation, namely, pumping over the tower cooler, was required.

TABLE 4

Effect of personnel factor on the bacterial count of milk

	Personnel					
	1	2	3	4	5	6
Av. plate count/ml.	8,700	16,000	67,000	8,100	272,000	87,000
No. of observations	22	24	5	7	1	3

In operating the open-combine system, cooling was delayed until the entire herd was milked. With the closed-combine and the cow-to-cooler systems, the cooling process began with the milking of the first cow and was continuous throughout the entire milking process.

SUMMARY

Four different systems of milking and milk-handling at the farm were compared, including an open-combine system, a closed-combine system, a conventional machine milking system and a cow-to-cooler combine milker installed in a

stanchion-type barn. The cow-to-cooler system involved delivery of the milk through a pipeline 200 ft. long which was cleaned throughout the study without dismantling.

Bacteriological analyses covered a period of 7 mo. and involved 484 observations. The results indicate that milk of acceptable quality can be produced by

TABLE 5

The effect of outside daily maximum temperature on the quality of milk produced

Temperature range	Group 1				Group 2			
	Open combine		Closed combine		Conventional		Pipeline cow-to-cooler combine	
	No. of observations	Count/ml.	No. of observations	Count/ml.	No. of observations	Count/ml.	No. of observations	Count/ml.
30–34			4	5,300			4	16,000
35–39	2	13,500	10	5,400	4	1,600	8	1,900
40–44	2	24,500	12	4,100			14	10,500
45–49	2	5,100	6	4,200	2	1,200	6	3,900
50–54	4	8,100	6	4,600	4	1,300	6	4,000
55–59	4	10,500	10	12,000	4	1,400	8	1,800
60–64			4	2,700	4	9,100		
65–69	12	12,000	8	5,600	8	2,400	12	19,000
70–74	12	12,500	12	7,300	6	4,300	18	7,500
75–79	24	10,500	24	5,100	26	5,000	18	10,500
80–84	20	15,000	24	8,700	20	7,000	22	10,500
85–89	24	17,500	7	6,700	6	15,500	26	15,000
90–91	6	7,800	2	3,700	2	6,100	6	4,500

use of any one of these systems. However, personnel may be an important factor in the production of quality milk using the cow-to-cooler system cleaned in place.

Variations in outside temperatures had very little effect on the bacterial count when the milk was cooled within 30 minutes. When cooling was delayed until the conclusion of the entire milking chore, there was a tendency toward a higher bacterial count when higher outside temperatures prevailed.

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MODIFIED RESAZURIN REDUCTION TEST FOR ESTIMATING FERTILIZING CAPACITY OF BULL SEMEN[1]

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Resazurin was reported by Erb and Ehlers (2) to show promise as a simple and rapid reduction test indicator for estimating fertilizing capacity of bull semen. The time required for semen to reduce resazurin to the pink endpoint compared with 60- to 90-day non-return rates (on a per semen sample basis) showed a highly significant correlation of -0.141 for 371 semen samples. Concentration was observed to be highly correlated with resazurin reduction time, with concentration showing no relationship to fertilizing capacity (3). Ehlers and Erb (4) standardized sperm concentration to 750,000 mm.^{3} before determination of resazurin reduction time. This method yielded a highly significant correlation of -0.517 between resazurin reduction time and non-return rate (72 semen samples).

The purpose of this experiment was to verify the results observed for sperm standardization and devise a more rapid method for compensating for the effect of varying concentration on resazurin reduction time.

PROCEDURE

Semen from 54 different bulls was used during the course of this study. The experimental period for Experiment 1 was 8 mo. at Evergreen Breeders Association (27 bulls) and 12 mo. for Experiment 2 at Northwest Co-op Breeders (27 bulls). All semen samples were routinely collected with the artificial vagina and selected for routine use on the basis of motility and previous knowledge of the bull's breeding performance by the technician of each organization. Dilution with egg-yolk citrate rarely exceeded 1 : 75, so that sperm per milliliter of diluted semen should not have been a limiting factor.

Concentration was determined by the hemocytometer method. Sixty- to ninety-day non-returns to first and second service were recorded for each sample by day of use after collection.

The resazurin test solution was made up fresh at 2- to 3-wk. intervals using 11 mg. of resazurin (from Aniline Dye Corp. in tablet form) per 600 ml. of *M*/7 phosphate buffer for studies at Northwest Co-op Breeders (Expt. 2) and 11 mg. of resazurin in 200 ml. of glass-distilled water for studies at Evergreen Breeders (Expt. 1). An incubation temperature of 37.5° C. was used, instead of 45° C.

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as previously described (2, 3, 4), to slow up the speed of the reaction and to prevent possible temperature shock.

At Evergreen Breeders (Expt. 1), the semen was standardized to approximately 750,000 sperm per mm.^{3} with phosphate buffer. Small culture tubes containing 0.1 ml. of resazurin test solution (11 mg. per 200 ml. of $M/7$ phosphate buffer) were placed in the water bath and 0.2 ml. of the standardized semen was pipetted directly. The mixture was agitated to insure complete mixing of dye and semen and then was layered with mineral oil. Time to reduce to a clear pink endpoint, except at the mineral oil-mixture junction, was recorded in minutes. The white endpoint was not used in these studies, since earlier work (2, 3, 4) indicated it had little predictive value as a test of fertilizing capacity.

A different procedure was used at Northwest Co-op Breeders (Expt. 2). Three-tenths ml. of resazurin test solution was used with 0.2 ml. of fresh unaltered semen. Thus, the same quantity of resazurin was used by each association. Incubation temperature was 37.5° C. It is important that this test be made on semen within 20 minutes post-collection and this was the procedure followed in this study. Statistical analyses were as outlined by Snedecor (5).

RESULTS AND DISCUSSION

Experiment 1. Resazurin reduction time was determined on 289 samples of semen standardized to 750,000 sperm per mm.^{3} at Evergreen Breeders. The average reduction time to pink was 7.26, with a standard deviation of 4.7 min. The correlation of resazurin reduction time with standardized concentration was highly significant (+0.238). This is in contrast to the correlation of −0.399 reported earlier by Erb *et al.* (3) and clearly indicates that the effects of concentration on the reduction time were not eliminated. It is believed that the addition of $M/7$ phosphate buffer was responsible, because reduction time (adjusted semen) plotted against original concentration showed a positive linear relationship, with reduction time increasing an average of 0.33 min. for each 100,000 increase in numbers of sperm per mm.^{3} Each of the 289 semen samples was then given a score based on standard deviation of reduction time plotted from the average regression line. The results as shown in table 1 reveal a high degree of selectivity, with a range in non-return of 63.4 and 56.8 per cent for the two highest grades, compared with 37.4 and 22.1 per cent for the two lowest grades.

One hundred and seventy-eight of these 289 semen samples representing 18 bulls were used for 18 or more first and second services. These samples were used for a correlation analysis, using the methods of covariance. The between-sample correlation of resazurin with non-return rate was −0.397 ($P < 0.01$), between bulls was −0.736 ($P < 0.01$) and for samples within bulls was −0.216 ($P < 0.01$). The correlation of concentration with non-return rate was −0.036. The partial correlation of resazurin with non-return rate independent of concentration was −0.419 ($P < 0.01$). The between-sample correlation of −0.397 is somewhat lower than the −0.517 reported earlier by Ehlers and Erb (4) on more limited data. Branton *et al.* (1), using a similar technique (adjusted to 300,000

sperm per mm.^{3}) for determining methylene blue reduction time, observed a correlation of -0.215 with non-return rate.

Experiment 2. While the above described resazurin method of grading semen shows considerable promise, it was felt that a simpler and faster method could be developed. The original method of Erb and Ehlers (2) did not show sufficient sensitivity at the higher concentration ranges, due to the too rapid reduction time to pink. One method of slowing reduction time was to reduce incubation temperature from 45 to 37.5° C. To guarantee more accuracy of reading the proper endpoint of the reduction (pink), 0.3 ml. of resazurin test solution made up in *M*/7 phosphate buffer was added with the 0.2 ml. of unaltered semen. While the quantity of resazurin used was unchanged from previous experiments, the total volume was increased by 0.2 ml. It was felt that the phosphate buffer also would decrease the speed of reaction and further increase sensitivity at the higher concentration ranges.

TABLE 1

Relation of semen grades as determined by resazurin reduction time on semen standardized to 750,000 sperm per mm.^{3} to non-return rate

Grade	Standard deviations from mean[a]	No. of samples	No. of first and second services	Non-return rate
				(%)
1	-2 or more	14	303	63.4
2	$-\frac{1}{2}$ to -1	54	1,325	56.8
3	$-\frac{1}{2}$ to 0	96	2,275	52.9
4	0 to $+\frac{1}{2}$	51	1,178	47.8
5	$+\frac{1}{2}$ to $+1$	38	664	51.8
6	$+1$ to $+2$	25	534	43.3
7	$+2$ to $+3$	7	99	37.4
8	over $+3$	4	149	22.1
Total		289	6,527	51.4

[a] Mean reduction time was 7.26 min. with standard deviation of 4.70 min. Minus signs indicate number of standard deviations below the mean regression line; plus signs the standard deviations above the mean regression line.

During a 1-yr. period, starting August, 1950, resazurin reduction time was determined on 780 semen samples using the method described above. Each of these samples was used for a minimum of 20 first and second services. Preliminary analysis revealed a curvilinear relationship between resazurin reduction time and concentration. Log concentration plotted against resazurin reduction time effectively corrected for the curvilinearity of the relationship. Resazurin reduction time changed -9.995 min. for each increase of 1.0 in log concentration. This relationship is shown in figure 1, where the solid black line represents the mean regression line for this set of data and each of the dotted lines above and below the mean represents one-half standard deviation (x resazurin = 5.17; s = 2.76) for resazurin reduction time. The numbers appearing between the subdivisions represent grades assigned to semen on the basis of resazurin reduction time and concentration. Non-return rates were calculated for each semen grade by day of use after collection and by breeds.

A total of 39,244 first and second services from 867 semen samples from 27 bulls of three breeds was summarized (table 2). The non-return rates by semen quality grades as determined from figure 1 show a range of 72.1 per cent for grade 3 down to 56.4 per cent for grade 12. The predictive value of this test was equally good for semen used the second and third days after collection. Semen was used the same day as collected only in one county by two inseminators; thus, numbers of inseminations are too few in several of the quality grade classes, but

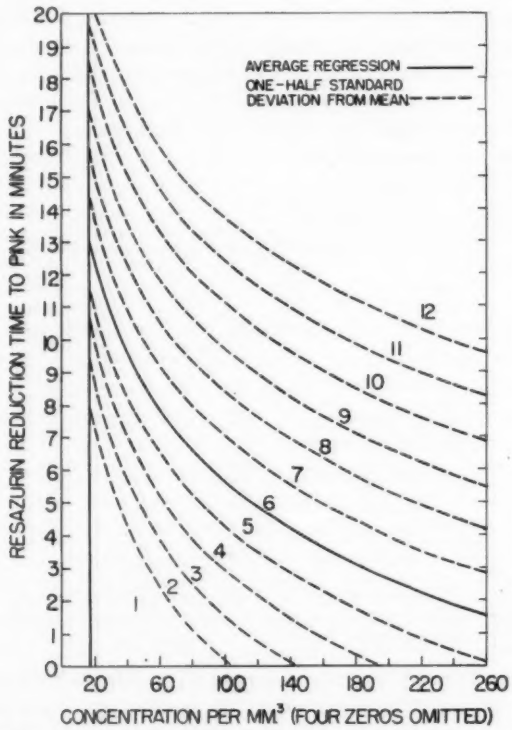

FIG. 1. Chart for correcting the effect of concentration on resazurin reduction time when separating semen into respective quality grades based on the resazurin reduction test.

for the most part show a decline in non-return rate for the poorer grades. Within breeds, Guernseys are uniformly lower at each grade than either Holsteins or Jerseys where there were 500 or more services available to calculate the non-return rate. The fertility level of all 27 bulls involved in this study was high, actually giving a very restricted range within which to predict fertilizing capacity. Only one bull had an average non-return rate below 60 per cent, and 11 of the 27 bulls exceeded 70 per cent.

Out of a total of 867 semen samples, 780 were used for 20 or more first and second services. These samples were used for the correlation analysis, using

TABLE 2

Non-return rates by semen quality grades as determined from figure 1

Semen quality grades	Day of use after collection								Day of use after collection							
	1		2		3		Total		1		2		3		Total	
	Serv.[a]	N.R.	Serv.[a]	N.R.	Serv.[a]	N.R.	Serv.[a]	N.R.	Serv.[a]	N.R.	Serv.[a]	N.R.	Serv.[a]	N.R.	Serv.[a]	N.R.
	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)
	Guernsey								*Holstein*							
1 and 2	8	62.5	5	60.0	1	0.0	14	57.1	2	100.0	35	71.4	33	81.8	70	77.1
3	40	85.0	165	72.1	4	50.0	209	74.2	52	72.5	465	72.9	271	69.0	788	71.7
4	439	70.8	1,676	65.9	325	68.3	2,440	67.1	308	72.1	2,083	70.0	668	67.4	3,059	69.6
5	900	70.9	3,910	67.5	954	66.5	5,764	67.9	403	73.9	2,320	69.2	1,071	69.3	3,794	69.7
6	772	67.2	3,322	65.9	571	61.3	4,665	65.6	233	71.2	1,662	70.2	837	64.3	2,732	68.5
7	558	69.4	2,398	64.6	581	61.1	3,537	64.8	135	68.1	878	67.4	379	61.2	1,392	65.8
8	251	70.1	1,069	62.9	214	62.1	1,534	64.0	31	74.2	199	70.9	115	69.6	345	70.7
9	87	64.4	431	62.4	42	33.3	560	60.5	4	75.0	100	66.0	96	64.6	200	65.5
10	28	64.3	116	56.0	2	50.0	146	57.5	9	55.6	66	66.7	20	45.0	95	61.1
11	18	77.8	89	59.6	2	50.0	109	62.4	.....	.....	.....	.....	.....	.....	.....	.....
12	63	60.3	244	55.3	49	61.2	356	57.0	7	42.9	45	53.3	.....	.....	52	51.9
Total	3,164	69.4	13,425	65.6	2,745	63.5	19,334	65.9	1,184	72.0	7,853	69.5	3,490	66.7	12,527	69.0
	Jersey								*Three breed total*							
1 and 2	1	0.0	9	55.6	.....	.....	.....	.....	11	63.6	49	67.3	34	79.4	94	71.3
3	79	77.2	358	73.2	136	65.4	573	71.9	171	78.4	988	72.9	411	67.6	1,570	72.1
4	253	74.7	1,537	73.6	499	70.1	2,259	73.1	1,000	72.2	5,296	69.8	1,492	68.5	7,788	69.8
5	369	73.4	2,056	71.5	795	67.9	3,220	70.9	1,672	72.2	8,286	69.0	2,820	67.9	12,778	69.2
6	134	79.9	709	74.6	253	67.6	1,096	73.6	1,139	69.5	5,693	68.2	1,661	63.8	8,493	67.5
7	25	68.0	119	70.6	9	66.7	153	69.9	718	69.1	3,395	65.6	969	61.2	5,082	65.2
8	5	80.0	19	68.4	.....	.....	24	70.8	287	70.7	1,287	64.2	329	64.7	1,903	65.3
9	1	100.0	17	64.7	.....	.....	18	66.7	92	65.2	548	63.1	138	55.1	778	62.0
10	.....	.....	.....	.....	.....	.....	.....	.....	37	62.2	182	59.9	22	45.5	241	58.9
11	.....	.....	.....	.....	.....	.....	.....	.....	18	77.8	89	59.6	2	50.0	109	62.4
12	.....	.....	.....	.....	.....	.....	.....	.....	70	58.6	289	55.0	49	61.2	408	56.4
Total	867	75.0	4,824	72.7	1,692	68.3	7,383	72.0	5,215	70.9	26,102	68.1	7,927	65.9	39,244	68.0

[a] = 1st and 2nd service insemination.

TABLE 3

Correlation summary

Source of variation	D.F.	Correlations					Partial correlation resaz. × non-return rate independent of log conc.
		Resazurin X		Non-return rate X			
		Conc.	Log conc.	Conc.	Log conc.	Resaz.	
Total	778	−0.473**	−0.657**	−0.032	−0.080**	−0.227**	−0.373**
Between breeds	1	−0.878	−0.739	+0.912	+0.789	−0.998*	−0.609
Samples within breeds	776	−0.468**	−0.662**	+0.005	−0.112**	−0.160**	−0.314**
Between bulls within breeds	22	−0.800**	−0.800**	+0.036	+0.047	−0.257	−0.366
Samples within bulls	753	−0.404**	−0.628**	+0.002	−0.144**	−0.145**	−0.305**

* Significant at 5% level of probability.
** Significant at 1% level of probability.

methods of covariance. These results, as shown in table 3, reveal that log concentration was highly significantly correlated with non-return rate in a negative direction for all samples, samples within breeds and samples within bulls. Resazurin reduction time correlated with non-return rate was – 0.227 for all samples ($P < 0.01$), – 0.257 between bulls and – 0.145 for samples within bulls. This is higher than the – 0.141 observed earlier by Erb and Ehlers (2), using a different method for determining reduction time. This is believed due to increased sensitivity at the higher concentration ranges, because average reduction time was doubled for this experiment.

The partial correlation between resazurin reduction and non-return rate, independent of concentration, was – 0.373 or nearly double the relationship for resazurin with non-return rate unadjusted for varying concentration. The partial correlation for samples within bulls was increased from – 0.145 to – 0.305.

The two studies reported herein do not lend themselves to combination, since the resazurin reduction time was not determined in the same manner. Taking into consideration variable dilution of semen with *M*/7 phosphate buffer at Evergreen Breeders (Expt. 1) which never would exceed 1 : 2 and on the average was considerably less than 1 : 0.5 in contrast to a 1 : 1.5 dilution at Northwest Co-op Breeders (Expt. 2), it would appear the average for Experiment 1 should fall somewhere in the quality grade 10 of figure 1. The bulls at this latter organization varied in non-return rate from 24.2 to 65.3 and showed a between-bull correlation of – 0.736 for resazurin reduction time compared with non-return rate.

Resazurin reduction time determined at 37.5° C. with 0.2 ml. of semen diluted 1 : 1.5 with *M*/7 phosphate buffer could be rapidly carried out routinely, since the average highly fertile bull with average concentration would reduce resazurin to pink in 5 min. or less. Concentration could be determined either photoelectrically or with the hemocytometer and the grade quickly determined by referring to a chart similar to figure 1. This test could be made on each sample collected, with no more than 15 min. lapse between collections. This test serves two purposes: (a) To estimate probable fertility of new stud bulls, and (b) to select the samples of higher fertility from bulls that have demonstrated satisfactory fertility through field use.

SUMMARY

Resazurin reduction time was determined for 1,156 semen samples from 54 bulls over a 12-mo. period and used by two cooperative breeding associations in Washington to breed 45,771 first- and second-service cows.

The method of determining resazurin reduction time was altered as follows: Incubation temperature was reduced from 45 to 37.5° C. Semen from one organization was standardized with *M*/7 phosphate buffer to 750,000 sperm/mm.^{3} and 0.2 ml. of the standardized semen was used, whereas the second organization determined reduction time for 0.2 ml. of fresh semen diluted 1 : 1.5 with *M*/7 phosphate buffer containing the resazurin dye. All reduction tests were started within 20 minutes after collection.

Resazurin reduction time for 289 semen samples from 27 bulls with standard-

ized sperm concentration was used for 6,527 first and second services. These bulls showed an average 60- to 90-day non-return rate of 51.4 per cent. The highest quality grade averaged 63.4 per cent and the lowest 22.1 per cent. The between-sample correlation of resazurin reduction time and non-return rate (178 samples with 18 or more first and second services) was -0.397 ($P < 0.01$) and was compared with -0.736 ($P < 0.01$) between bulls, and -0.216 ($P < 0.01$) for samples within bulls.

Resazurin reduction time for 867 semen samples from 27 bulls for 0.2 ml. of fresh semen diluted 1:1.5 with $M/7$ phosphate buffer was used for 39,244 first and second services for an average non-return rate of 68.0 per cent. The bulls ranged from 59.3 to 75.9 per cent non-return rate. Concentration affected resazurin reduction time curvilinearly, at the rate of 9.995 min. decrease in resazurin reduction time for each increase of 1.0 in log concentration. A curve was drawn and semen quality grades assigned from 1 through 12, which gave a range in non-return rate of 71.9 per cent for grades 1 and 2 compared with 56.4 per cent for grade 12. The correlation between log concentration and non-return rate for samples within bulls was -0.144 ($P < 0.01$) and was $+0.002$ for concentration and non-return rate for samples within bulls ($n = 753$). Resazurin reduction time and non-return rate showed a correlation of -0.227 ($P < 0.01$), -0.998 ($P < 0.05$), -0.160 ($P < 0.01$), -0.257 and -0.145 ($P < 0.01$) for between samples ($n = 778$), between breeds ($n = 1$), and samples within breeds ($n = 776$) between bulls within breeds ($n = 22$) and samples within bulls ($n = 753$), respectively; the partial correlations independent of concentration were -0.373 ($P < 0.01$), -0.609, -0.314 ($P < 0.01$), -0.366 and -0.305 ($P < 0.01$), respectively. This test offers a simple, rapid method for estimating fertilizing capacity of bulls and samples within bulls.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

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BOOK REVIEWS

464. Milk Testing. J. G. DAVIS. Dairy Industries, Ltd., London, England. 260 pp. 15s. 1951.

A wide variety of physical, chemical and bacteriological tests as they apply to milk and closely related products is presented. Naturally the viewpoint is that of the British Isles, but those in other areas will find much that is applicable and of interest. A very interesting and worthwhile section is that concerned with organization and operation of a dairy control laboratory. Material on design, preparation of materials, determination of costs and other items not commonly easily available are presented. References to the literature providing background for various tests are given, adding considerably to the probable usefulness of the book for many. Indexing, organization and printing seem well done.

F. E. Nelson

465. Advances in Enzymology, vol. 13. Edited by F. F. NORD. Interscience Publishers, Inc., New York. 413 pp. $8.50. 1952.

The chapters included are: Localization of enzymes in cytoplasm, by H. Holter; Some aspects of the application of tracers in permeability studies, by Hans H. Ussing; La biosynthèse induite des enzymes (Adaptation enzymatique), by Jacques Monod and Melvin Cohn; Genetic formulation of gene structure and gene action, by G. Pontecorvo; Adenosine triphosphate and the structural proteins in relation to muscle contraction, by D. M. Needham; Hyaluronidases, by Karl Meyer and Maurice M. Rapport; Certain aspects of intermediary metabolism of glutamine, asparagine and glutathione, by Heinrich Waelsch; Stoichiometric inhibition of chymotrypsin, by K. A. Balls and Eugene F. Jansen; and The comparative biochemistry of nitrogen fixation, by Perry W. Wilson. F. E. Nelson

466. Vitamin Methods, vol. II. Edited by PAUL GYÖRGY. Academic Press, Inc., New York. 740 pp. $14.50. 1951.

The chapters included are: General aspects of small animal experimentation, by N. B. Guerrant; The animal vitamin assays, by C. I. Bliss and Paul György; Laboratory diagnosis of human vitamin deficiencies, by James Hazlitt Jones; Clinical signs of malnutrition, by Norman Jolliffe; and Statistical methods in vitamin research, by C. I. Bliss. Three chapters supplementary to volume I are included: Physical methods of vitamin assay, by Eric T. Stiller, covers methods involving ultraviolet adsorption spectra and fluorimetric determinations. Chemical methods of vitamin assay by Paul György, Saul H. Rubin and Elmer De Ritter, covers vitamin A, β-carotene, vitamin E, niacin, niacinamide, panthenol and pantothenic acid, choline and ascorbic acid. Microbiological methods in vitamin research, by Lemuel E. Wright, Helen R. Skeggs, Saul H. Rubin and Elmer De Ritter, is concerned with determinations of vitamin B_{12} and panthenol. Nearly all of the material should be very valuable to those doing nutritional work. F. E. Nelson

467. The chemistry and technology of food and food products, vols. 1, 2 and 3. 2nd ed. Edited by MORRIS B. JACOBS. Interscience Publishers, Inc., New York, N. Y. 2,580 pp. $12.00, $15.00 and $15.00. 1951.

The presentation in the 3 volumes is divided into 6 parts: Fundamentals, Unit Operations and Processes, Sanitary and Quality Control, Foods (description of the individual types), Preservation and Production. The 52 chapters are written by specialists in the different areas. Two chapters deal directly with milk and other dairy products and numerous references to these products are found in other chapters. Where so many authors are concerned, one cannot expect completely uniform treatment of the individual subjects. Neither of the chapters on milk products employs as many references to original literature as might be desirable for a somewhat condensed presentation. The complete subject index at the end of volume 3 is very useful, but the abridged indices of volumes 1 and 2 are not very satisfactory for reference purposes. Probably the greatest value of these books to those in the dairy industry, aside from a survey of some areas in the industry with which one may not be familiar, is to show how other food industries handle their similar problems. Those who furnish dairy products to other food industries could gain insight into how these products are used in the other industries.

F. E. Nelson

BUTTER

O. F. HUNZIKER, SECTION EDITOR

468. Extraneous matter in butter—a pressing problem. E. A. GIERMAK, Marketing Assn. of

America, Chicago. Am. Dairy Prod. Rev., **14,** 7: 24, 25. July, 1952.

During the past 5 yr. approx. 200 seizures of butter have been made by the Federal Food & Drug Adm. This represents about 3.7% of total seizures. Shipments adjudged guilty of violation can be considered a total loss. In addition, heavy fines have been levied. Approx. 95% of extraneous mattter is in the cream on arrival at the creamery and 5% enters during processing. The conditions at the creamery can be more readily controlled than can those affecting the cream prior to arrival at the plant. In-coming raw materials must be tested for sediment to detect parties supplying products with excessive extraneous matter. Producers must be informed of results and cream rejections made when regulations are violated. The control of extraneous matter is an important problem to the butter industry.

T. J. Claydon

469. Effect of non-washing on commercial creamery butter. D. R. L. Arnott, V. W. Greene and D. L. Gibson. Can. Dairy Ice Cream J., **31,** 4: 31–33, 44. Apr., 1952.

Benefits apparently can be derived from dispensing with the use of wash water in butter manufacture (increase of overrun, saving of time and saving of chilled water). The results indicate that there was a greater percentage of 40-score butter among the unwashed churnings. In a study of split churnings of butter held at 20° F., there was little difference between the flavor of washed and unwashed butter after 45-d. storage. Commercial churnings of unwashed butter stored at 10° F. were scored 50% 40-score, and 44% 39-score after 102 d. of storage. The quality of the cream influenced the scores of washed and unwashed butter in a similar fashion. Bacteriologically there were no essential differences between the butters after storage. The curd content of washed butter averaged 0.8% and that of unwashed butter was found to be 1.5%. Unwashed butter showed an increase in overrun of 0.75% over washed butter. pH values of the 2 butters, both fresh and in storage, were similar. The non-washing of commercial creamery butter is commercially feasible but needs further study.

H. Pyenson

470. Manufacturing butter by the "Gold'n Flow" process. V. H. Neilsen, Iowa State College, Ames. Am. Dairy Prod. Rev., **14,** 6: 16–18, 20–22. June, 1952.

A detailed description is given of the continuous butter-making process used by the Wapsie Valley Creamery Co., Cedar Rapids, Ia., since 1949. The product moves through the plant without contact with human hands. Fat losses are less than in conventional churning. Composition is more uniform and more accurately controlled. The butter texture is close and, due to fine moisture dispersion, bacterial keeping quality is high. The method is preferably one for large-scale operation.

T. J. Claydon

471. Assessment of the Fritz continuous butter-making machine for Canadian use. J. A. Pearce, D. Rose and H. Tessier, Nat. Research Council, Ottawa, Can. Can. J. Technol., **30,** 7 and 8: 167–177. 1952.

Some 350 trials, conducted over a 4-yr. period on a Westphalia model of a Fritz continuous butter-producing machine have indicated that this machine is not suited to the production of butter for the Canadian market. Great difficulty was experienced in producing butter of legal moisture content unless the machine was operated at reduced capacity. Fat losses in the buttermilk were high and no satisfactory method of incorporating salt was devised.

O. R. Irvine

472. Standardizing the color of butter. A. Reinart and R. W. Brown. Can. Dairy Ice Cream J., **31,** 5: 31–33, 78. May, 1952.

In this method the butterfat is separated from the cream by using the Minnesota Reagent. The separated fat is dissolved in gasoline and the shade of natural color of fat is compared with color standards of known intensities of color. Each standard corresponds to a certain amount of artificial butter color. Comparison of these standards with the gasoline solution of the fat sample gives the amount of artificial butter color to be added to the cream to give the butter a standard shade of color.

H. Pyenson

473. Butter coloring tablet and method of coloring butter. L. Adler, H. A. Reinhardt and E. E. Ponath (assignors to Dyestuffs and Chemicals, Inc.). U. S. Patent 2,600,381. 11 claims. June 17, 1952. Official Gaz. U. S. Pat. Office, **659,** 3: 679. 1952.

A fat-soluble dye and a starchy material are compressed into tablets which easily disperse in the aqueous phase of butter.

R. Whitaker

474. Effect of iron content on quality of Quebec butter. J. P. Julien and G. Martin. Can. Dairy Ice Cream J., **31,** 7: 36–38, 50. July, 1952.

The Fe content of the 68 butter samples analyzed ranged from 0.32 to 1.65 ppm. with an average of 0.905 ppm. No significant relation has been found between the Fe content and the increase in the peroxide index during the storage. In the oxidized samples containing less than 0.15 ppm. Cu, an Fe content over 1.00 ppm. caused a very significant increase in the peroxide index in many cases.

H. Pyenson

475. A glassine-aluminum foil wrap for print butter. A. H. White. Can. Dairy Ice Cream J., **31,** 6: 32–33. June, 1952.

The results of the trials showed that the special water-resistant no. 20 glassine paper was a very satisfactory substitute for light-weight parchment when laminated to aluminum foil for packaging print butter. Glassine is reported to cost less than light-weight parchment.

H. Pyenson

476. Butter warming receptacle. B. H. Simpson. U. S. Patent 2,595,010. 5 claims. April 29, 1952. Official Gaz. U. S. Pat. Office, **657,** 5: 1524. 1952.

Butter, removed from the refrigerator, is softened and made spreadable by placing it in this device, which serves as a dish and is designed to fit on top of an electric toaster or other heating means. R. Whitaker

477. Sanitary butter dispenser. H. E. Young and G. V. Farnham (assignors of 42.5% to H. E. Young, 42.5% to G. V. Farnham, 4% to R. E. Anderson, 4% to B. W. Cox, 2% to N. B. Spahr, and 5% to B. R. Waller and H. F. Hayes, jointly). U. S. Patent 2,603,868. 5 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 894. 1952.

Pats of butter for table use are dispensed 1 at a time by pressing a cutting blade against a 0.25-lb. stick of butter R. Whitaker

478. Butter gun. W. Van Guilder (assignor to General Mills, Inc.). U. S. Patent 2,603,867. 2 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 893. 1952.

A device for extruding any desired amount of butter for table or cooking use, consists of a plunger which forces the product out of a shell and against a cutting blade. R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

479. Experiments on cheddar cheese ripening. T. R. Freeman, Kentucky Agr. Expt. Sta., Lexington. Am. Dairy Prod. Rev., **14,** 7: 2–4. July, 1952.

The effect of ripening temperature on cheese quality was investigated by curing cheese at the following temperatures: (a) 12 wk. at 60° F.; (b) 4 wk. at 60° F. then 8 wk. at 40° F.; (c) 12 wk. at 50° F.; (d) 8 wk. at 40° F. then 4 wk. at 60° F.; (e) 12 wk. at 40° F. The cheese ripened under method *b* was the most satisfactory. In 12 wk. a good quality cheese with "mild" to "medium" flavor was produced by the latter procedure. "Acidity" was the most common flavor criticism. Non-fat dry milk solids was compared with skimmilk for standardizing. The differences in cheese quality or yield were not significant, but the manufacuring time was shorter with the NDMS. T. J. Claydon

480. Manufacture and finishing of Canadian cheddar cheese. Part I—practical notes on cheese manufacture. J. F. Robinson; and **Part II—finishing commercial and exhibition cheese.** J. M. Bain. Can. Dairy Ice Cream J., **31,** 4: 74–83. Apr., 1952. H. Pyenson

481. Nine years' work on control of extraneous matter in cheese. O. R. Irvine and W. H. Sproule. Can. Dairy Ice Cream J., **31,** 5: 36–38, 48. May, 1952.

The authors review the development of test methods for extraneous matter in cheese by college and government personnel in Canada. The effort to reduce the excessive extraneous matter in cheese has been successful. The cheese now can be shipped to U. S. or other markets. The program has reached the point where the cheese is subject to continuous inspection and was effective in pointing up the need for better production methods on the farm and in the factory. H. Pyenson

482. Cheese press. R. Miollis (assignor to The Borden Co.). U. S. Patent 2,604,036. 8 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 940. 1952.

A multiple vertical cheese press is described. R. Whitaker

483. Uniform processed cheese. B. M. Zakariasen, Land O'Lakes Creameries, Inc., Minneapolis, Minn. Food Eng., **24,** 7: 65–67, 131–132, 133. July, 1952.

Land O'Lakes Creameries, Inc., produces over 100,000 lb. of processed cheese daily. In achieving optimum quality, 4 basic factors are stressed: (a) A varied and sizable bulk cheese supply; (b) full services of a skilled blender; (c) a smoothly engineered processing system; (d) careful integration of quality control with production. Good blending requires from 3–12 different kinds of cheese including "current" cheese (not over 30 d. old), "short-held" cheese (1–6 mo. old) and "storage" cheese (over 6 mo. old). Acidity is an important factor. A typical blend for 475 lb. of American-type processed cheese includes 75 lb. current, 50 lb. acid short-held, 200 lb. slightly-acid short-held, 100 lb. slightly-alkaline short-held and 50 lb. aged cheddar. Selected cheeses are tempered 72 hr. at 70° F. to stabilize moisture content. Processing operations are in timed sequence. Cheeses are unhooped and prepared for weighing. Grinding and cooking operations follow. Emulsifier, salt, color and water are added during cooking. The processed cheese is packaged in plastic form and cooled. A quality testing program is applied throughout the process, and spot lots of the finished product are subjected to further tests. T. J. Claydon

484. The use of whey protein in process cheese production. J. Babad, J. Avidor, M. Sharon-Shtrikman and A. Grünpeter, Agr. Research Sta., Rehovoth, Israel. Food Technol., **6,** 4: 143–147. 1952.

Laboratory experiments on the coagulation of whey protein by heat showed that the addition of 0.5% $CaCl_2$ to whey was effective in stabilizing certain properties of the whey protein so that at pH levels below 5.6 coagulation temperature is increased, settling rate is decreased and consistency of whey protein is improved. The addition of Ca to whey evidently protects the protein from denaturation and increases the "bound water" content of the coagulated protein. Other cations apparently have the same effect as Ca.

In pilot plant studies 20–50% of whey protein prepared by addition of Ca to whey was blended with ground hard cheese with an emulsifying solution added. The finished product was reported to have a desired texture and flavor. E. R. Garrison

485. Cheese package. F. H. Cherepow and G. W. Forcey (assignors to Marathon Corp.).

U. S. Patent 2,606,120. 6 claims. Aug. 5, 1952. Official Gaz. U. S. Pat. Office, **661,** 1: 225. 1952.

A flexible heat-sealable, grease-resistant, moisture-proof wrapper for cheese is described which is permeable to CO_2 and is easily peeled from the cheese. R. Whitaker

486. Process for making nonmilk fat stable cheese. H. J. PETERSEN. U. S. Patent 2,604,405. 4 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 1043. 1952.

A process-type of cheese is made as follows: Skimmilk rennet curd is pressed for about 14 hr. and aged at low temp. for about 6 wk. The aged cheese is ground or shredded, pasteurized and blended at 150–180° F. with such edible oils, other than milk fat, as peanut, cottonseed, coconut, lard, etc. R. Whitaker

487. Manufacture of cottage cheese and other cultured milk products. N. C. ANGEVINE. Can. Dairy Ice Cream J., **31,** 5: 34–35, 50. May, 1952.

The author discusses, under 19 headings, the important factors affecting the manufacture of uniform high quality cottage cheese. H. Pyenson

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

488. Method of cencentrating milk. G. L. CUNNINGHAM (assignor to The Commonwealth Eng. Co.). U. S. Patent 2,602,745. 5 claims. July 8, 1952. Official Gaz. U. S. Pat. Office, **660,** 2: 432. 1952.

Milk is concentrated by freezing it to a slush in a series of freezing chambers. The product cascades from 1 chamber to another, increasing in concentration as the ice crystals are removed and moved upward to the preceding chamber. R. Whitaker

489. Milk product method of manufacture. R. E. MEADE (assignor to Western Condensing Co.). U. S. Patent 2,602,746. 4 claims. July 8, 1952. Official Gaz. U. S. Pat. Office, **660,** 2: 433. 1952.

A spray-dried milk product of high water-absorbing properties for use in bread, made by blending whey and skimmilk, acidifying at a temp. of 185–280° F., settling the curd, removing the supernatant liquid and drying, is described. R. Whitaker

490. Milk product process of manufacture. R. E. MEADE (assignor to Western Condensing Co.). U. S. Patent 2,602, 747. 2 claims. July 8, 1952. Official Gaz. U. S. Pat. Office, **660,** 2: 433. 1952.

A spray-dried milk product of high water-absorbing properties for use in bread is made by acidifying whey to pH 4.0–5.6, neutralizing to pH 6.0–9.0 and heating to 185–280°. It then is blended with hot skim and held at that temp. for 2.5–30 min. before concentrating, adjusting the pH to 4.0–5.6, neutralizing to pH 6.0–9.0 and drying. R. Whitaker

491. Milk substitutes. S. G. WIECHERS (assignor to de Nederlandsche Centrale Organisatie Voor Toegepast-Natuurwetenschappelijk Onderzoek). U. S. Patent 2,604,403. 9 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 1043. 1952.

The albumin, globulin and lactose from electrodialyzed whey, with the solids containing less than 4% ash, are blended with an edible fat and casein to form a liquid infant food substitute for milk. R. Whitaker

492. Can holder and punch. M. L. GROB. U. S. Patent 2,601,939. 1 claim. July 1, 1952. Official Gaz. U. S. Pat. Office, **660,** 1: 136. 1952.

An apparatus holds and punches 2 holes in cans, such as used for evaporated milk, 1 for pouring and 1 for an air vent. R. Whitaker

493. Milk can holder. J. F. CONNOR. U. S. Patent 2,597,295. 1 claim. May 20, 1952. Official Gaz. U. S. Pat. Office, **658,** 3: 769. 1952.

A holder for a can of evaporated milk which facilitates punching the can and pouring contents is described. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

494. Dilution procedures for plate counts on dry milk. A. H. WHITE, Dept. of Agr., Ottawa, Can. Sci. Agr., **32,** 1: 19–25. 1952.

Comparative plate counts made on roller- and spray-dried skimmilk powders and on spray-dried whole milk powders showed that the use of 0.1 *N* LiOH rather than H_2O resulted in counts being greatly reduced. The pH of the reconstituted milk in 0.1 *N* LiOH (approx. 10.6) was thought to be responsible for this reduction. Tempering diluents to 50° C. did not result in reduced counts, as compared to lower temps. Varying types of organisms characteristic of dry milk were shown to be equally affected by LiOH. O. R. Irvine

495. Bacterial spoilage in process cheese. E. G. HOOD AND K. N. SMITH, Dept. of Agr., Ottawa, Can. Sci. Agr., **31,** 12: 530–540. 1951.

Methods are described by which gas-producing clostridia were isolated from spoiled process cheese and suspected samples of Cheddar and blue cheese. 64 out of 100 samples of non-fat dry milk solids and 47 out of 51 samples of raw milk Cheddar cheese gave evidence of containing clostridia. *Cl. pasteurianum* and *Cl. sporogenes,* as well as 2 similar species, produced the defect when inoculated into experimental process cheese. *Cl. tertium, Cl. perfringens* and 2 additional species did not.

Heavy inoculations, pH values above 5.40 and NaCl concentrations below 3.5, all operated to favor spoilage. O. R. Irvine

496. The lactic streptococci in Edmonton milks and creams. G. A. NELSON AND H. R. THORNTON, Univ. of Alberta, Edmonton, Can. Can. J. Technol., **30,** 5: 130–135. 1952.

Of 3,000 isolates from 59 samples of raw milk, approx. 8% were typical lactic streptococci, 2% fecal streptococci and 8.5% alkali formers. Of

the typical lactics, 96% were *S. lactis* and 4% *S. cremoris*. No typical lactic streptococci were isolated from over 50% of the above milks and none from 10 samples of freshly HTST-pasteurized milk, only one of which soured normally. No isolations of this group were made from 2 udder milks or 1 foremilk, either before or after souring. Typical lactic streptococci predominated in 15 sour churning creams. It is concluded that *S. lactis* in its natural habitat does not flourish in this climate. (From authors' abstract) O. R. Irvine

497. The nature of fermented milks. R. G. Morey, Brook Hill Farms, Inc., Chicago, Ill. Milk Dealer, **41,** 7: 46, 64–66. Apr., 1952.

The flavor of fermented milk varies with the type of organism and the milk used. Fermentations are of 2 main types—acid fermentation and a combination of acid and gas fermentation. The best known fermented milks used in different countries of the world are: lactic buttermilks, bulgarian buttermilk, yogurt, acidophilus milk, kefir and kumiss. The latter 2 are examples of the combination gas and acid fermentation. Bulgarian and acidophilus milks have gained widespread use in this country. The properties of these milks are described and directions are given for their preparations. C. J. Babcock

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

498. Recherches sur la biochimie des composés phophorés du lait (Investigations on the biochemistry of phosphorus-containing compounds of milk). H. Simmonet and J. Sternberg. Lait, **32:** 272–282. May–June, 1952.

Utilization of radioactive P in the mammary glands of a lactating dog and a number of lactating rats was studied. A quantity not exceeding 25% of such P was recovered in the milk in the form of phosphoprotein. This amount varies as a function of time and appears to depend upon the conversion of mineral phosphate into limited amounts of a precursor which ultimately is transformed into caseïn. It is proposed that this precursor is phosphopyruvate which is transformed into phosphoserine. The mechanism by which this precursor is transformed into casein appears to depend closely upon the level of plasma ribonucleate. Study of protein constituents of the lactating gland showed that it contains a protein richer in P than may be considered as another intermediate of the precursor. S. Patton

499. Process for the recovery of milk proteins. S. R. Hoover (assignor to the United States of America, as represented by the Secretary of Agriculture). Application 175,850. July 8, 1952. Official Gaz. U. S. Pat. Office, **660,** 2: 480. 1952.

Roller or spray milk powder is acidified with a mineral acid to pH 4.1 and the coagulated protein, after removal of the whey, is extracted several times with a salt solution, such as NaCl, Na_2SO_4, etc., having a strength about 0.2–0.5% at pH 3.9–4.7. When dried, the protein is free of lactose and contains about 91% of the original protein. The lactose can be recovered from the combined filtrates. R. Whitaker

500. Lactalbumin of improved taste for human consumption. W. J. Pratt and F. H. Tinkler (assignors to Wyeth Inc.). U. S. Patent 2,606,-181. 4 claims. Aug. 5, 1952. Official Gaz. U. S. Pat. Office, **661,** 1: 267. 1952.

Whey is decaseinated and defatted by heating to 75–150° F. at pH 4.0–5.2 and filtering with a filter aid. The lactalbumin in the clear filtrate is coagulated by heating to and holding at 195–205° F. until it can be filtered and washed without filter aid. R. Whitaker

501. Chemicals from milk. A staff-industry collaborative report. M. L. Kastens, Assoc. Editor, and F. A. Baldauski, Chemical Div., Sheffield Farms Co., Inc., Norwich, N. Y. Ind. Eng. Chem., **44,** 6: 1257–1268. 1952.

The production of casein, milk-protein powder, caseinates, whey proteins, lactose, protein hydrolyzates, tyrosine and other special chemical products derived from milk at the Sheffield Farms, Norwich, N. Y. plant is described. 60,000 gal. of the raw materials, skimmilk and whey, can be processed daily. Whole milk is separated and skimmilk usually goes directly to the casein machine. Casein is manufactured continuously by precipitation with HCl, draining off the whey, washing and drying the curd. The casein is sold for industrial uses or further processed into milk-protein powder, caseinates or protein hydrolyzates. The whey removed from the casein operation is heated to precipitate the whey-protein fraction, which is used for protein hydrolyzate or animal feed manufacture. The clarified whey is concentrated, the lactose crystallized from it, separated and dried. With the increasing costs of milk and its seasonal supply, continuous improvements in processing, and improved recoveries must be made. B. H. Webb

502. Etude du lait dont la caséin est floculée par le chauffage (Study of milk from which the casein is flocculated by heating). M. Lust. Lait, **32:** 241–258. May–June, 1952.

Studies were made to obtain the best and simplest method of preparing milk possessing "the Utrecht anomaly" in which the casein is flocculated in very fine, stably dispersed particles. Such milk, when properly prepared and fortified with starch or hydrolyzed starch derivatives, was found excellent for infant feeding. In general, the procedure involves treatment of milk with 2% of calcium lactate or 2.5% of calcium gluconate and 2% of starch. The milk is brought to a boil for approximately 1 min. with agitation. This results in optimum thickening and fineness of the flocculation. Specific directions for treatment of raw, pasteurized, homogenized and evaporated milks are given. Directions for adjusting total solids and adding various carbohydrates also are presented. S. Patton

503. Hydrolysis of casein with sulphur dioxide. J. W. Pedersen and B. E. Baker. Nature, **169,** 4309: 928. 1952.

Heating casein, SO_2 and water in a sealed tube to 100° C. indicated that SO_2 is an effective hydrolyzing agent with less destruction of tryptophane than with H_2SO_4 in the absence of reducing agents. R. Whitaker

504. Deux procédés simples pour dépister l'addition de lait de vache au lait de femme (Two simple procedures to detect the addition of cow's milk to mother's milk). F. Alison. Lait, **32:** 258–262. May–June, 1952.

The difficulties and advantages of numerous methods for detecting the adulteration of mother's milk with cow's milk are discussed. In an attempt to improve on existing methods, the author has developed 2 simple procedures which, in principle, are as follows: (a) To 10 drops of milk to be tested in a small tube are added 2 drops of saturated $CuSO_4$ solution. The tube is stoppered, inverted and returned to the upright position. Pure mother's milk remains homogenous. Adulteration with cow's milk provokes a clearly evident granulation of the proteins on the side of the tube. The lower limit of reliable sensitivity is 20% admixture of cow's milk. (b) To 2 ml. of milk to be tested in a test tube are added 4 ml. of 0.4% $CdSO_4$ solution. The tube is placed in a boiling water bath for 1 min, then allowed to cool for a few min. At this point, pure mother's milk remains homogenous and opalescent. The presence of cow's milk in the sample is evident from gross coagulation, where adulteration is heavy, or the formation of a white ring at the surface in the case of slight adulteration. The lower limit of sensitivity of this test is 5%. The tests are applicable irrespective of whether raw, pasteurized, boiled, sweetened or unsweetened condensed milks are used as adulterants. S. Patton

505. Fireproof, waterproof, flexible sheeting resistant to penetration by chemical warfare vesicant agents. W. H. Aiken and A. L. James (assignors to the U. S. A., as represented by the Secretary of War). U. S. Patent 2,606,131. 5 claims. Aug. 5, 1952. Official Gaz. U. S. Pat. Office, **661,** 1: 257. 1952.

A flexible sheeting is made resistant to the penetration of chemical warfare vesicants by a laminated layer of ammoniacal formaldehyde hardened casein. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

506. Power washing centrifugal separator. J. R. Orelind (assignor to International Harvester Co.). U. S. Patent 2,603,413. 4 claims. July 15, 1952. Official Gaz. U. S. Pat. Office, **660,** 3: 698. 1952.

A separator bowl is designed to be washed without dissembling. A centrifugal valve is provided which allows a cleaning solution to flow rapidly through the bowl to flush out the slime and clean the entire bowl and discs. R. Whitaker

507. Commercial ice cream freezer. H. F. Swenson. U. S. Patent 2,604,307. 10 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 1018. 1952.

An ice cream freezer of the horizontal type built into a cabinet has the front end extending through the front wall of the cabinet and is driven by a pulley at the rear of the cabinet. R. Whitaker

508. Processing and cooling of canned foods. Some heat transfer problems. V. H. Hemler, D. V. Alstrand, O. F. Ecklund and H. A. Benjamin, American Can. Co., Maywood, Ill. Ind. Eng. Chem., **44,** 6: 1459–1465. 1952.

The rate of heat penetration into foods heated in sealed cans in a retort can be changed by improper venting of the retort. Temperature variations throughout the retort occur when air is mixed with steam in the retort. The resulting uncertain heat distribution has a substantial effect on the sterilizing value of the process on those products which heat rapidly and which need only a short process time. Long heating lags have only a slight effect on the process sterilizing value of products that heat by conduction. Pressure maintained on cans after the steam is shut off prevents the contents from boiling and mixing in the cans. If boiling is permitted by release of external pressure, cooling is rapid and the sterilizing value is reduced somewhat. B. H. Webb

509. Problem of waste disposal in the dairy industry. G. T. Stander. Can. Dairy Ice Cream J., **31,** 6: 56, 60–62. June, 1952; and **31,** 7: 48–49. July, 1952.

The methods available for treatment are (a) purification by irrigation and lagooning, (b) disposal into water courses, (c) chemical coagulation and (d) treatment by biological methods, which include adding mixture with domestic sewage and subsequent treatment at the sewage disposal works, treatment by the activated sludge process, treatment of milk wastes by anaerobic digestion and treatment by biological filtration.

Milk processing effluents fall into 3 distinctly different classes: (a) Milk washings, (b) whey washings and (c) waste waters from creameries. H. Pyenson

510. Progress in plant design and construction and a look into the future. G. R. Johnson, Pace Assoc., Chicago, Ill. Milk Dealer, **41,** 9: 40–41, 52. June, 1952.

HTST pasteurization has produced a significant reduction in the floor area formerly required for batch pasteurization. Sanitary pumps have allowed single level operations and a consequent simplification of building plans. Conveyor systems have affected the relationship of various parts of a plant. The use of fork lift trucks and palletizing for handling both supplies and finished products, automatic equipment, loading

and unloading technics and devices, heating systems, electrical control installations, bulk farm pickup, 3A standards, permanent sanitary lines, etc. also have influenced plant layout and design. Regardless of construction or materials, a plant must be designed around the nucleus of the processing operations it is to enclose, the flow of raw materials to the operations and the flow of finished products to distribution facilities. To achieve this, the various functions of a plant are separated into: (a) Processing, including receiving of raw milk; (b) cold storage and distribution facilities, including truck storage and servicing; (c) warehousing, receiving, storage and handling of materials and supplies; (d) facilities for mechanical equipment and utilities, including engine room and shop areas; and (e) administrative, office and public areas. Characteristics of future plants will include milk processed into its various products by fully automatic technics, whole milk sold in unrefrigerated cans off the grocer's shelf, ice cream produced in a streamlined sequence operated by several men and electrical controls and butter and cheese manufactured by fully automatic equipment. There already is a trend toward larger plants and "push button" operation. C. J. Babcock

511. Bimetallic thermometer for use in the dairy industry. A. H. LAMB (assignor to Weston Electrical Instrument Corp.). U. S. Patent 2,603,091. 3 claims. July 15, 1952. Official Gaz. U. S. Pat. Office, **660,** 3: 603. 1952.

A small indicating thermometer of the bimetallic type having the dial made to the exact dimensions of a sanitary cap is described. The thermometer is installed in a sanitary pipe tee and held in place by a regular sanitary pipe hex nut. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

512. Creative processing. Food Eng. Staff. Food Eng., **24,** 7: 92–94. July, 1952.

At Rochester Dairy Co-op. in Minn. 1 million lb. of milk daily are processed into 35 different products. This outstanding growth is related to 2 basic policies: (a) Ready development of promising products which meet local market conditions of the consumer; (b) intensive sales promotion relating to the new products. 17 different ice cream mix powders are made, including "Sof-Serv" containing 6% fat. Plastic cream also is one of the company's important products. Rochester Dairy has been a pioneer in making incentive payments for quality milk from its 4,000 producers. T. J. Claydon

513. Work simplification. C. H. BROADED, Fisher Flouring Mills Co., Seattle, Wash. Am. Dairy Prod. Rev., **14,** 7: 18–20. July, 1952.

Application of work simplification technics may reduce by 50% or more, time required for certain operations. It is not a speed-up system but a reduction of wasted energy. Three basic principles are: (a) Eliminate all unnecessary motions of men or material; (b) make the necessary motions as short as possible; (c) use the best possible sequence of motion in order to prevent duplication, back tracking and cross travel. Operations first are analysed by application of flow, process, machine and operator charts. Improvement in working performance of the operator is accomplished by reduction in expended motion and proper placing of tools and equipment. It is best to apply work simplification principles by teaching foremen, who then can instruct the workmen. T. J. Claydon

514. More sales through merchandising. A. H. JOHNSON, J. Walter Thompson Co. Milk Dealer, **41,** 8: 106–122. May, 1952.

Merchandising in its broader marketing sense is the recognition of basic trends and opportunities—basic changes in population and its composition, in consumer needs and purchasing power, in standards of living and buying habits—in such a way that every selling and advertising dollar is made to produce more sales. The present economic conditions are favorable to improved standards of living and diet. Basic changes in population, number of children, family formation, education, home equipment and consumer buying habits all offer favorable opportunities for sales expansion of dairy products. Therefore, a substantial expansion in consumption of dairy products definitely is possible. C. J. Babcock

515. Changing the pattern of food distribution. P. S. WILLIS. Milk Dealer, **41,** 9: 112–118. June, 1952.

A discussion of the changes that have taken place in food distribution is followed by these predictions: (a) The pattern of distribution will continue to change; (b) competition will be very severe; (c) new products will fight for a position on the shelf and for the consumer dollar; (d) non-food items in large numbers will find their way into grocery stores; (e) advertised brands will continue their growth, particularly those brands which are supported by strong advertising and merchandising, and which carry a potential reasonable dollar profit opportunity for distributors; (f) the grocery bill will grow bigger and bigger because of increased population and because people generally are eating better; (g) labels and packages will become more attractive and provide more descriptive and useful information; (h) self-service will continue to grow, and gradually fresh meats, fruits and vegetables and perishable items will move more and more into self-service; (i) the outlook for volume business is good, but for profits it is not so good. C. J. Babcock

516. Salesmen's auto expense allowances. Anonymous. Milk Dealer, **41,** 9: 98–101. June, 1952.

A survey on salesmen's auto expense allowances includes reports received and tabulated from 218 companies with a combined total of 4,769 automobiles. Tables are presented showing a break-

down on the ownership of cars, basis of payment, rates of allowance and mileage and the operating costs of company-owned cars. C. J. Babcock

517. Research the key to progress G. E. Holm. Can. Dairy Ice Cream J., **31,** 7: 27–28, 60. July, 1952.

Only through direction of its development and the constant adaptation and use of new knowledge can the dairy industry attain efficiency and stability. There is a great opportunity for the dairy industry to promote, through research, utilization of a large proportion of milk solids-not-fat for human food. Research work in the field of nutrition has demonstrated that the non-fat solids of milk are not only a unique but a necessary supplement to our diet to assure good nutrition. H. Pyenson

518. Dairy industry trends in the United States. P. H. Tracy. Can. Dairy Ice Cream J., **31,** 7: 33–35, 44. July, 1952.

The more important trends and problems are: (a) tank haulage of milk (improved sanitation and lower hauling costs); (b) tank storage of milk on the farm; (c) 6-d. processing week; (d) consolidation of plants; (e) higher HTST pasteurization of milk; (f) milk beverages low in fat and high in milk-solids-not fat; (g) disappearance of public health trade barriers; (h) palletizing in milk plants; (i) quality problem of psychrophilic organisms in milk and cream; (j) special ice cream stores and food stores that make own ice cream; (k) soft ice milk manufacture; (l) HTST mix pasteurization; (m) use of liquid sugars in ice cream; (n) increased use of molded and preformed ice cream items; (o) increased use of dried non-fat milk solids; (p) use of powdered creams; (q) development of country-style cottage cheese; (r) popularity of cream cheeses; and (s) development of continuous butter making equipment. H. Pyenson

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

519. Stability of carotene concentrates. H. L. Mitchell, W. G. Schrenk and R. E. Silker. Kansas Agr. Expt. Sta., Manhattan. Ind. Eng. Chem., **44,** 5: 1123–1124. 1952.

Studies on free-flowing concentrates were made with 3 carriers to determine the effect of the addition of various stabilizing substances. Expeller cottonseed and soybean meals and unconverted rice bran showed some ability to improve carotene retention when they were mixed with extracted plant lipides or alfalfa meal.
B. H. Webb

520. Moisture determination of silage, hay and grain. A. I. Magee and W. Kalbfleisch, Central Exptl. Farm, Ottawa, Can. Sci. Agr., **32,** 3: 117–126. 1952.

Seven methods of determining moisture were studied and are described. Three were rapid drying methods, 2 humidity methods, while the others utilized $CaCl_2$ and pressure. The rapid drying methods and the $CaCl_2$ method all require the use of a scales but are quite accurate. The pressure method is of use over a very narrow range and the humidity methods are not precise for hay, but provide an indication of the moisture present in grain. O. R. Irvine

521. Feed supplement. H. R. Stiles (assignor to Commercial Solvents Corp.). U. S. Patent 2,603,567. 9 claims. July 15, 1952. Official Gaz. U. S. Pat. Office, **660,** 3: 741. 1952.

Ammonia is caused to react with the sugar from such crude materials as blackstrap molasses, hydrol syrup, wood sugar syrup and citrus and distillers molasses to form a nitrogenous material suitable for feeding to cows and other ruminants.
R. Whitaker

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

522. Dehorning shear apparatus. J. Black (assignor to Armour and Co.). U. S. Patent 2,606,340. 12 claims. Aug. 12, 1952. Official Gaz. U. S. Pat. Office, **661,** 2: 373. 1952.

An apparatus for dehorning cattle is provided with nozzles for discharging hot and cold water at time of cutting. R. Whitaker

523. Protective sheath for cows' teats. F. E. Brown and P. Martin. U. S. Patent 2,604,092. 8 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 957. 1952.

A bag-like protective sheath for covering cows' teats is composed of flexible elastic material with the neck portion restricted and designed to hold the device in place. R. Whitaker

524. Animal weaner. A. W. Barker. U. S. Patent 2,602,421. 4 claims. July 8, 1952. Official Gaz. U. S. Pat. Office, **660,** 2: 342. 1952.

A harness attaches to the head of calves, etc. for weaning purposes. It carries a source of electrical energy and a means for delivering a shock when a ring-operated switch is activated by the animal. R. Whitaker

525. Uterus-retaining truss and harnessing means therefor. J. Payton. U. S. Patent 2,606,552. 4 claims. Aug. 12, 1952. Official Gaz. U. S. Pat. Office, **661,** 2: 432. 1952.

A device for preventing cows from everting the uterus through the vulva is described.
R. Whitaker

526. Stall with retaining gate. J. D. Youngman. U. S. Patent 2,601,845. 2 claims. July 1, 1952. Official Gaz. U. S. Pat. Office, **660,** 1: 110. 1952.

A mechanism for simultaneously releasing cattle from a row of stanchions is described.
R. Whitaker

527. Restraining device for cattle. C. L. Norton. U. S. Patent 2,601,623. 1 claim. June 24, 1952. Official Gaz. U. S. Pat. Office, **659,** 4: 1087. 1952.

To a rigid vertical upright placed between each cow stall about opposite the cow's shoulder is attached a flexible loop, parallel with the floor

and about shoulder high. If the cow moves from the center of the stall, the loop is flexed by the side of the cow and the animal is urged by contact with the loop to remain in the center of the stall. R. Whitaker

528. Milking machine. E. B. THOMPSON (assignor to Globe Hoist Co.). U. S. Patent 2,606,523. 3 claims. Aug. 12, 1952. Official Gaz. U. S. Pat. Office, **661,** 2: 424. 1952.

Details cover the construction of a milking machine including teat cups, reservoir for the milk and means of operating the device. R. Whitaker

529. Pipe line milking system. S. DAILY, R. M. LATHROP AND O. E. HINTZ (assignors to International Harvester Co.). U. S. Patent 2,606,524. 6 claims. Aug. 12, 1952. Official Gaz. U. S. Pat. Office, **661,** 2: 425. 1952.

A system of pipes for permanent milking machine installation in a milking barn is described. R. Whitaker

530. Milking machine hanger. L. F. FASCHING. U. S. Patent 2,602,621. 1 claim. July 8, 1952. Official Gaz. U. S. Pat. Office, **660,** 2: 399. 1952.

A bracket for attaching to the stall for holding a milking machine is described. R. Whitaker

531. Inflations for milkers. T. W. MERRITT AND C. A. THOMAS (assignors to Babson Bros. Co.). U. S. Patent 2,604,873. 4 claims. **July 29, 1952.** Official Gaz. U. S. Pat. Office, **660,** 5: 1243. 1952.

A teat cup for a milking machine is described. The flexible inner pulsator has several longitudinal ribs. R. Whitaker

532. Milk strainer. L. F. ZERNICKE. U. S. Patent 2,601,079. 2 claims. June 17, 1952. Official Gaz. U. S. Pat. Office, **659,** 3: 863. 1952.

A design for a milk strainer has the filtering medium protected by a dome-shaped member from the force of milk poured into the strainer. R. Whitaker

533. Container and closure therefor. W. E. CRAMER. U. S. Patent 2,604,226. 1 claim. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 995. 1952.

A milk can having a cover of the umbrella type which slides down on the outside of the can neck and has a curved flange which rests on the can shoulder when the cover is in place is described. R. Whitaker

534. Liquid cooler. J. M. BRUNSTAD. U. S. Patent 2,600,474. 5 claims. June 17, 1952. Official Gaz. U. S. Pat Office, **659,** 3: 702. 1952.

A funnel-shaped milk cooler for farm use, refrigerated by a small compressor is described. R. Whitaker

535. Apparatus for agitating refrigerated liquids. H. G. ROBATEL (assignor of ½ to K. S. Cate). U. S. Patent 2,604,308. 2 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 1018. 1952.

A tank of constantly agitated refrigerated water cools milk in cans on the farm. The cans are placed on a platform which tips slightly on a fulcrum causing a pendulum type of agitator suspended from the can cover to stir the milk and facilitate cooling. R. Whitaker

536. Milk can handling device. K. A. E. LUOMALA. U. S. Patent 2,606,732. 1 claim. Aug. 12, 1952. Official Gaz. U. S. Pat. Office, **661,** 2: 483. 1952.

A telescoping mast for facilitating lowering cans of milk in and out of cooling tanks is described. R. Whitaker

537. Apparatus for filling milk cans by vacuum, with means for straining and cooling said milk. E. G. REDIN, K. E. ORLANDER AND Z. A. E. JOHANSSON (assignors to Aktiebolaget Manus). U. S. Patent 2,603,396. 2 claims. July 15, 1952. Official Gaz. U. S. Pat. Office, **660,** 3: 692. 1952.

A series of milk cans fitted with tight covers is held in a chilled water bath. The cans are connected in series by pipes. The can at 1 end is attached to a vacuum supply and the can at the other end is attached to the milk reservoir of a milking machine. The incoming pipe in each can extends to the bottom, while the outlet is at the full mark. A strainer is installed in the milk line between the milker and 1st can. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

538. The use of butter oil in the manufacture of ice cream. E. J. SMITH, Meadow Gold Dairies, Washington, D. C. Ice Cream Trade J., **48,** 7: 52. July, 1952.

Butter oil containing 99.8% fat and not more than 0.1% moisture packed in 25-lb. tins may be used in ice cream. Up to 60% replacement of this fat from sweet cream may be made. W. H. Martin

539. Frozen confection. K. R. BROWN (assignor to Atlas Powder Co.). U. S. Patent 2,602,749. 3 claims. July 8, 1952. Official Gaz. U. S. Pat. Office, **660,** 2: 433. 1952.

An ice cream improver consists of a straight-chain acid containing 12-20 C atoms and terminal acyl radicals of the general formula $R_1OC\text{-}H_2(CH_2OCH_2)CH_2OR_2$, where R_1 and R_2 are acyl radicals. R. Whitaker

540. Process for stablizing foodstuff and stabilizing composition. N. BLIHOVDE (assignor to The Borden Co.). U. S. Patent 2,604,406. 9 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 1043. 1952.

An ice cream stabilizer consisting of from 1 to 12 parts of a cellulose ether and 1 part of Irish moss is described. R. Whitaker

541. Emulsified ice cream composition. E. I. VALKO (assignor to E. F. Drew & Co., Inc.). U. S. Patent 2,606,838. 5 claims. Aug. 12, 1952. Official Gaz. U. S. Pat. Office, **661,** 2: 510. 1952.

An ice cream emulsifier consists of a diester of a polyethylene glycol having a mol. wt. of 300–4000 with a higher fatty acid of at least 10 C atoms and a fatty acid having 2–4 C atoms. R. Whitaker

542. Modified Babcock procedures for testing ice cream mix. J. A. Meiser and P. S. Lucas. Can. Dairy Ice Cream J., **31,** 5: 39–40, 74. May, 1952.

Eight variations of the Babcock test, designed for use on ice cream mixes, are compared on the basis of laboratory results. The Mojonnier test was used as a standard for accuracy. The tests studied were (a) sulfuric-glacial acetic acid; (b) Illinois non-acid; (c) Minnesota; (d) mixed perchloric and acetic acids; (e) Nebraska; (f) Kniaseff; (g) Pennsylvania; and (h) modified Pennsylvania.

The authors conclude that 6 of the testing procedures used failed to produce average algebraic and arithmetical procedures within the range of 0.2%. Also all 8 testing procedures produced extreme variations that fell outside the proposed limit of accuracy. Further perfection of any one of the original modifications studied is desirable instead of proposing a new method. H. Pyenson

543. Volume shrinkage of ice cream during storage. E. L. Thomas, W. B. Combs and S. T. Coulter. Can. Dairy Ice Cream J., **31,** 4: 36–42. Apr., 1952.

Ice cream from mixes heated at 185° F. for 10 min. or 165° F. for 30 min. shrank more than that from mixes heated for either shorter or longer periods at either temperature.

The addition of colostrum to increase the globulin content tended to increase shrinkage. Heat treatments of ice cream mixes which yield the maximum tendency towards shrinkage also coagulate significant amounts of the serum protein, but do not coincide with the heat treatment necessary to cause complete coagulation of these proteins. It appears that dry ice-induced shrinkage is primarily physical in nature and involves disruption of the ice cream structure through internal pressure changes associated with alternate absorption and desorption of CO_2. Shrinkage trends were similar using spontaneous and vacuum-treatment methods. Shrinkage was not accelerated by jolting of ice cream. H. Pyenson

544. Device for holding ice cream cans in position. D. W. Auch. U. S. Patent 2,601,935. 7 claims. July 1, 1952. Official Gaz. U. S. Pat. Office, **660,** 1: 135. 1952.

A clamp holds cans of ice cream immovable while the product is being dispersed from a cabinet. R. Whitaker

545. Cheaper by the dozen. Anonymous. Ice Cream Trade J., **48,** 7: 28, 29, 90. July, 1952.

Bulk ice cream to take home in dips is a new method of selling bulk ice cream introduced by Abbott's in Philadelphia. The package is a special sanitary clear white container made to the Milk Control Board specifications. It is molded from clean, virgin wood pulp, chemically treated to kill bacteria and fungi, and waxed. The box holds 12 individual "hand-dipped" portions of bulk ice cream of uniform size. A no. 24 dipper is used. The carton fits the freezing section of tray compartments of most home refrigerators. Each dip of ice cream weighs about 1.9 oz. and the average gross weight of a filled tray is 24.5 oz. The tray weighs 1.5 oz. W. H. Martin

546. Whistle stop no. 1. Anonymous. Ice Cream Trade J., **48,** 7: 34, 89. July, 1952.

Something new in the way of retail outlets for ice cream was introduced at 3334 Lawrence Ave. on Chicago's north side. The front of the shop is painted in the form of a diesel engine front in orange and red, complete with flashing head lights and surrounded by electrically operated semaphores and signal lights. Directly behind the front is a glass-enclosed kitchen and frozen custard stand, where chefs dressed as engineers prepare the servings. Sidewalk customers are served directly from ticket windows, on each side of the kitchen. In the dining room is a 30-stool counter with miniature electric train track circling the inside edge on a riser about 9 in. above the counter. When orders are given the waitress punches out the items on a ticket which goes to the kitchen. When the food is ready, it is placed on the Lionel miniature diesel freight train, the whistle blows, the "all aboard" is given and an electronic control system dispatches the train to 1 or more stops. W. H. Martin

547. Vegetable fat frozen desserts. V. M. Rabuffo. Ice Cream Trade J., **48,** 7: 20–22, 24, 94–97. July, 1952.

Questions are asked and answers given in so far as they can be made at this time relative to present status of vegetable fat desserts. The IAICM has resolved that the integrity of ice cream must be maintained and that suitable regulations governing labeling and advertising of products containing vegetable fats should be adopted and enforced. In recent weeks most Chicago, Ill., ice cream manufacturers have introduced vegetable-fat frozen desserts of high- and low-fat content. In Texas most of the vegetable-fat products contain 10–12% fat and must be labeled Mellorine. California permits the manufacture of vegetable-fat desserts, but provides that they cannot be made in a plant where ice cream is made. Vegetable-fat frozen desserts in Chicago sell at wholesale for $1.18–$1.30/gal., in pt. packages, compared to $2.20/gal. for pt. packages of ice cream. Some ice cream manufacturers believe that the industry will eventually produce (a) a high-fat, low-overrun ice cream to sell at a premium price; (b) a standard commercial grade of ice cream; (c) a low-fat dairy product such as ice milk and (d) a vegetable-fat frozen dessert of low- or highfat depending on conditions in the local market. W. H. Martin

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

548. Milk control planning for civil disaster. G. E. McCALLUM AND J. D. FAULKNER, USPHS. Pub. Health Repts., **67,** 7: 635–639. 1952.

An enemy air attack on a large population center is likely to destroy or at least disrupt the operations of milk processing and cold storage plants along with transportation and utility services. Civil defense authorities of potential target cities must develop plans and procedures to be followed after such a disaster to conserve and protect the city's milk supply, to provide for its processing in outlying areas and to insure milk distribution to those needing it. Due to the increased possibilities for disease transmission during civil disasters the sanitary control of fluid milk and milk products is important. Emphasis should be placed on control of the pasteurized supply rather than the producers or country receiving stations. Plans also should be made for instructing the public how to pasteurize or sterilize raw milk in the event that pasteurizing facilities are inadequate and raw milk is distributed. Provision should be made for the substitution of concentrated milk products if the flow of raw milk into the city is disrupted.

Milk and milk products directly contaminated with chemical warfare agents must be destroyed. Products in sealed containers and plant facilities exposed to radioactive mists must be decontaminated by washing or scrubbing exposed surfaces according to instructions applicable to the agent used. Procedures must be devised for the protection of processing operations against possible contamination with biological agents by enemy agents.

Further research is believed necessary on (a) the use of chemical preservatives and sterilizing agents to replace the heat treatment of milk and (b) rapid field tests for detecting the contamination of milk and milk products with radioactive, chemical or biological agents.

Any plans for milk control services must be coordinated with other civil defense plans for probable disaster for each specific area.

D. D. Deane

549. Bulk handling of milk on the farm. H. E. CALBERT, Univ. of Wis. Milk Dealer, **41,** 7: 106–112. Apr., 1952.

In bulk handling of milk on the farm as the milk is produced it either is piped or carried from the barn or milking parlor into the milkhouse where it passes through some type of strainer and then into a bulk holding tank. In most instances the holding tanks are refrigerated and equipped with an agitator to enable rapid cooling. Due to the refrigeration capacity of the holding tanks and the agitation, the milk is cooled so rapidly that it is possible to mix night and morning milk with little danger of bacterial growth. The milk is picked up at the farm by a tank truck or tank trailer. It is pumped through a rubber or plastic hose from the bulk holding tank into the transport. The pump usually is carried on the tank truck. The bulk handling of milk on the farm originated on the West Coast about 13 yr. ago. It is being tried on a practical or semi-experimental basis in at least 12 or 13 states. The advantages in using this system are: (a) It saves labor; (b) handling and transportation costs are reduced; (c) aids in production of a high quality milk. Use of this system in Wisconsin will require modifications or changes in the system and in regulations and control measures that are concerned with production of and care of high quality milk. These problems are concerned with measuring the milk for volume or weight, sampling it for fat and for bacteriological control purposes and with sanitary measures that must be taken with the pickup equipment such as hose, pipelines, etc.

C. J. Babcock

550. Pour la pasteurisation obligatoire du lait de consommation (Toward compulsory pasteurization of market milk). J. PIEN. Lait, **32:** 262–275. May–June, 1952.

This is the 3rd and final paper of a series which presents the case for compulsory pasteurization of milk. S. Patton

551. Fresh milk canned aseptically for shipment to overseas markets. J. STAMBAUGH. Can. Dairy Ice Cream J., **31,** 4: 34–35, 44. Apr., 1952.

Through the use of special milking, handling, sterilizing techniques, the Martin aseptic canning process and a series of special processing methods developed by International Milk Processors, Inc., milk now is being canned on a commercial scale. The product keeps for 4–6 mo. without refrigeration. Its color, taste and appearance are those of fresh pasteurized milk. The present selling price is slightly above that for fresh milk in the United States. H. Pyenson

552. Siphon for filling milk cans. W. J. TAMMINGA (assignor to Wiz-Products, Inc.). U. S. Patent 2,604,294. 5 claims. July 22, 1952. Official Gaz. U. S. Pat Office, **660,** 4: 1014. 1952.

A valve on the bottom of a siphon tube for controlling the flow of milk into cans is described. R. Whitaker

553. Defoamer for bottling machines. E. R. ANDRE (assignor to Ex-Cell-O Corp.). U. S. Patent 2,604,247. 9 claims. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 1000. 1952.

To remove the foam which may form on milk bottled in the Pur-pak type of paper milk bottle filling machine, an additional station is provided directly after the filling operation, at which time the foam is eliminated by blowing the foam into an area of reduced pressure. R. Whitaker

554. Preformed bottle cap. R. H. HORNING (assignor to Oswego Falls Corp.). U. S. Patent 2,604,223. 1 claim. July 22, 1952. Official Gaz. U. S. Pat. Office, **660,** 4: 994. 1952.

A cover-all bottle cap composed of a molded synthetic material is described. R. Whitaker

555. Process for preparing a sterilized cream for preservation in sealed containers. V. NELSON (assignor to Food Machinery and Chemical

Corp.). U. S. Patent 2,603,568. 7 claims. July 15, 1952. Official Gaz. U. S. Pat. Office, **660,** 3: 741. 1952.

Milk is forewarmed to develop maximum heat stability and evaporated to form a concentrate which then is blended with normal cream in such proportions that the blend may be separated to form a secondary cream of from 15–30% fat and containing 10% MSNF. The secondary cream then is canned and sterilized. R. Whitaker

556. Accurate, sanitary milk dispensing. E. D. White, Atlanta, Ga. Food Eng., **24,** 7: 124. July, 1952.

An accurate and sanitary dispenser for milk and related fluids has been engineered by L. M. Leather's Sons of Athens, Ga. A precision electric timer controls the size of the portion delivered. Special construction features contribute to improved sanitation. Constant refrigeration is employed and the dispenser is stated to more than meet the code of the U. S. Public Health Service. T. J. Claydon

557. New Haven dairy finds automatic vending opens outlets and boosts volume. Anonymous. Milk Dealer, **41,** 7: 40, 41, 54–56. Apr., 1952.

The New Haven Dairy of New Haven, Conn., is operating 45 automatic vending machines located in industrial plants, educational institutions, the YMCA, a bank, an insurance co., a newspaper office, a local utility co., an auto showroom, and gas stations. These 45 machines are dispensing milk at a rate better than 1 million containers/yr. The machine dispenses 0.5 pt. of milk and chocolate milk drink priced at 10¢. Seven out of every 10 of the firm's vendors now average 100 or more daily sales. About 80% of the vending outlets are 5-d. stops, with the balance requiring Saturday service. The dairy views automatic vending as one of the most profitable milk marketing mediums that have come along in the last few years. C. J. Babcock

558. Dispensing milk container. J. P. Jones (assignor to Dairy Specialties, Inc.). U. S. Patent 2,601,399. 3 claims. June 24, 1952. Official Gaz. U. S. Pat. Office, **659,** 4: 1024. 1952.

Two sealed openings are provided in a paper milk container of the Pure-Pak type which are so arranged as to permit removal of the cream from the creamed milk if desired. R. Whitaker

559. Plastic milk carton holder. C. A. Olson (assignor of 50% to R. S. Hilliard). U. S. Patent 2,600,911. 1 claim. June 17, 1952. Official Gaz. U. S. Pat. Office, **659,** 3: 816. 1952.

A rigid plastic holder for paper milk bottles made to prevent distortion of the container when handled in the home is described R. Whitaker

560. Container with water shedding top construction. C. G. Preis (assignor to American Can Co.). U. S. Patent 2,601,945. 4 claims. July 1, 1952. Official Gaz. U. S. Pat. Office, **660,** 1: 137. 1952.

An improvement of the Canco-type of paper milk bottle consists of having the pouring lip raised slightly above the top of the container, thus shedding water and protecting against undesirable foreign material. R. Whitaker

MILK SECRETION

V. R. SMITH, SECTION EDITOR

561. A new galactose-containing compound from mammary glands. R. Caputto and R. E. Trucco. Nature, **169,** 4312: 1062. 1952.

A progress report indicates that a polysaccharide-like compound is formed in the mammary gland, which may be important in determining the exact synthesis of lactose. The compound is demonstratable by means of chromatography and labelled carbon-14 tracer experiments. R. Whitaker

562. Sugar chromatography of mammary gland extracts. F. H. Malpress and A. B. Morrison. Nature, **169,** 4313: 1103. 1952.

A number of reducing substances have been demonstrated which may be concerned with the synthesis of lactose, but these are not the same as those noted in Abs. 561. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

563. Fluoridated milk. Anonymous. Milk Dealer, **41,** 9: 49, 56–58. June, 1952.

According to J. E. McKee, at Calif. Inst. of Technology, milk, rather than public water supplies, may be the best vehicle for using fluoride as an antidental caries weapon. The arguments in favor of milk as a fluoride carrier are so enticing that some action should be taken by research agencies either to confirm or discredit them on the basis of sound fundamental data. The use of milk for this purpose involves questions of assimilation of milk-borne fluorides, quantities and varieties of daily consumption, effects of fluoridation on the palatability and nutritive value of milk, universal application, cost and reception by the public. Tests lead to the assumption that fluorides added to milk would be available for assimilation as readily as the fluorides added to drinking water. Any detrimental effects of fluorides upon the palatability and nutritive value of milk remain to be demonstrated. The cost is infinitesimal, $0.000,005/qt. C. J. Babcock

564. Age changes in relation to the ingestion of milk, water, coffee and sugar solutions. C. M. McCay, F. Lovelace, G. Sperling, L. L. Barnes, C. H. Liu, C. A. H. Smith and J. A. Saxton, Jr. J. Gerontol., **7:** 161–172. 1952.

Various diets were evaluated with respect to their effect on life span of weaned rats. One group of rats was fed pasteurized milk supplemented with various trace elements and cod liver oil. Three other groups were given 10% sucrose, coffee and water, respectively, as their only beverage. These latter groups received an adequate mixed diet with sugar added to that of the coffee and water groups. Growth for all groups

was similar with no differences in total life span. X-ray evidence showed more calcification in the group consuming milk alone than in the other groups. Milk tended to keep rats at the lowest level of body weight throughout life, while sucrose solution kept them at the highest. S. Patton

565. Your consumers' concerns concern you. Zoe E. Anderson, Natl. Dairy Council, Chicago, Ill. Ice Cream Trade J., **48,** 7: 40, 42, 86–87. July, 1952.

In answer to the question, do we need fats in our diets, the author points out that fats are our most concentrated form of food energy, supplying about 1/5th of the energy available from our food supply. Fat also improves the use our bodies make of protein, reduces the body's need for certain of the B-complex vitamins and makes it possible to eat fewer calories without experiencing hunger and fatigue. Certain nutrients which are necessary to body functioning are naturally found associated with fat in our food, such as vitamins A, D, E and K and certain fatty acids, which are essential to good health. Flavors in butterfat enhance its own value and improve the taste of all foods with which it is used; it is highly digestible and can be used in the diet of humans under some conditions where other fats are not well tolerated.

Fat-free and low-fat diets have been recommended for treatment of certain types of heart ailments, because animal fats contain cholesterol and cholesterol can be formed from either animal or vegetable fats in the body. Since cholesterol, when deposited in the inner walls of the blood vessels, may cause hardening of the arteries, recommendations for low-fat and fat-free diets have been made; most research workers are agreed that in the normal individual, the cholesterol in the diet does not influence the blood levels of this material, since if we eat less cholesterol the body manufactures more. For certain individuals a low-cholesterol diet may be advisable and there is a possibility that restricted fat intake may help keep blood cholesterol level down.

Reducing diets have been low in fat content for various reasons, none of which is truly valid. Studies at Michigan State College show that weight can be satisfactorily lost, a diet more strictly adhered to and fatigue and hunger eliminated when a reducing diet contains equal amounts, by weight of protein, fat and carbohydrates.

The sugar content of ice cream posed a nutritional problem because it supplies nothing but energy and because sugar has been implicated as the cause of tooth decay. Sugar in ice cream is combined with the many milk nutrients and it has important nutritional value in addition to its energy value. Research to help clarify the contributing causes of dental decay and the importance of sound nutrition in preventing tooth decay now is underway. W. H. Martin

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

566. Modern cleaning techniques. Anonymous. Milk Dealer, **41,** 7: 43, 58. Apr., 1952.

The Hastings Co-operative Creamery, processing about 35,000 lb. of milk daily, reports a modern sanitation system has greatly reduced costs and is more convenient. No washing powder is used in the plant. A special liquid cleanser and a liquid sanitizer are located in tanks in the supply room. The liquids are pumped from this central location to points of use. Where it is necessary to put the cleaner into the water first, as in washing sanitary pipes, separator parts, etc., a stainless steel metering device is used, automatically measuring out the exact amount of cleanser. Both cleanser and sanitizer lines are connected to the can washer through an automatic electric control unit on the washer. C. J. Babcock

567. Permanent sanitary pipeline cleaning. F. F. Fleischman, Jr., Corning Glass Works, Corning, N. Y. Milk Dealer, **41,** 7: 130–135. Apr., 1952.

The results of experimental work in permanent sanitary pipeline cleaning at Michigan State College, Cornell Univ., Univ. of Georgia, and the Univ. of Ill. are reviewed. Much of this work is with glass tubing, which shows considerable promise as a substitute for metal milk pipes in dairy plants. Bacteriological evidence indicates glass tubing can be cleaned in an assembled position and maintained in a sanitary condition. Other factors which may enhance its use are (a) visible examination of the interior glass tubing is possible and (b) it appears free from microscopic surface irregularities; therefore, it is less liable than metal milk pipe to accumulate milk film. There are between 85 and 100 commercial installations of glass piping under the surveillance of either university research laboratories and/or local or regional health authorities. There have been no unfavorable reports from any of these installations. At H. P. Hood and Sons, Inc., Boston, Mass., data collected over a period of 5 yr. show use of permanent glass pipe lines between pasteurizing units and bottling machines definitely has reduced their positive coliform count to a low level. A recommended cleaning procedure is given for in-place cleaning.
C. J. Babcock

568 Use of field tests in evaluating detergents. J. L. Minkin, N. Y. State Pub. Health Environmental Sanitation Field Training Center, Buffalo, N. Y. Pub. Health Repts., **67,** 7: 650–652. 1952.

Simple tests are described for evaluating dishwashing detergents as to their solubility, foaming ability, control of water hardness, wetting ability, emulsification of fats and ability to deflocculate and dissolve proteins. They can be made using a 0.5-pt. bottle and a teaspoon with visual observation of the results. Small differences in detergents can not be detected but the

material can be classified as poor, fair, good or excellent.

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